Interaction of Cyclooxygenase Isoenzymes, Nitric Oxide, and Afferent Neurons in Gastric Mucosal Defense in Rats

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ABSTRACT

The cyclooxygenase (COX)-2 inhibitors 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2(SII)-furanone (DFU) (0.02–2 mg/kg) and N-[2-(cyclohexyloxy)-4-nitrofenyl]methanesulfonamide (NS-398) (0.01–1 mg/kg), the COX-1 inhibitor 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole (SC-560) (0.05–5 mg/kg), and dexamethasone (1 mg/kg) were studied in rats challenged with intragastric acid (300 mM HCl). All compounds induced severe gastric damage when rats were treated concurrently with the inhibitor of constitutive and inducible nitric oxide (NO) synthase Nω-monomethyl-l-arginine methyl ester (L-NAME) (3 or 40 mg/kg). DFU and NS-398 caused significantly less damage in rats receiving the selective inhibitor of inducible NO synthase N-(3-aminoethyl)benzylacetamide (1400W) (0.3 mg/kg). The COX-1 inhibitor SC-560 induced moderate damage in the acid-challenged stomach even without suppression of NO, but damage was aggravated by l-NAME. The COX-3 inhibitor phenacetin (400 mg/kg) did not injure the gastric mucosa despite suppression of NO. Furthermore, DFU, NS-398, SC-560, and dexamethasone caused severe injury in the acid-challenged stomach of rats pretreated with capsaicin to ablate afferent neurons. The mucosal damage induced by the COX-1 inhibitor, the COX-2 inhibitors, and dexamethasone in l-NAME- or capsaicin-treated rats was reversed by coadministration of 16,16-dimethyl-prostaglandin E2 (2 × 8 ng/kg). Gross mucosal damage was paralleled by histology. Our results support the concept that endogenous NO, prostaglandins, and afferent neurons act in concert in the regulation of gastric mucosal integrity. The prostaglandins necessary for mucosal defense in the face of NO suppression, and afferent nerve ablation can be derived either from COX-1 or COX-2. The data do not propose a protective role for a phenacetin-sensitive COX-3. Our findings suggest that not only COX-1 but also COX-2 has important functions in the maintenance of gastric integrity.

Various systems exist that contribute to gastric mucosal defense such as prostaglandins (PGs), nitric oxide (NO), and afferent neurons. In the acid-challenged stomach of normal rats, short-time pretreatment with indomethacin to inhibit prostaglandin biosynthesis, Nω-monomethyl-l-arginine (l-NAME) to inhibit NO formation, or capsaicin to ablate afferent neurons and deplete sensory neuropeptides did not cause mucosal injury. In contrast, indomethacin induced damage in rats treated concurrently with l-NAME. Furthermore, after indomethacin administration in capsaicin-pretreated rats, l-NAME induced widespread, hemorrhagic necrotic damage. From these findings, it was suggested that NO, prostacyclin, and sensory neuropeptides act in concert in the modulation of gastric mucosal integrity (Whittle et al., 1990).

Endogenous prostaglandins are generated from arachidonic acid. Three isoenzymes of cyclooxygenase (COX), COX-1 (Vane, 1994), COX-2 (Xie et al., 1991), and recently COX-3 (Chandrasekharan et al., 2002), have been described that catalyze the conversion of arachidonic acid to the prostaglandin endoperoxide H2 (PGH2), the key reaction in prostaglandin biosynthesis. COX-1 is constitutively expressed in most tissues (O’Neill and Ford-Hutchinson, 1993) and was suggested to yield the prostaglandins involved in “housekeeping” functions. In contrast, levels of COX-2 are usually low or undetectable under resting conditions (Kargman et al., 1996) but rapidly increase under the influence of proinflammatory or mitogenic stimuli (Raz et al., 1989; Kujubu et al., 1991). In the gastric mucosa of experimental animals and humans, high levels of COX-1 and low levels of COX-2 mRNA and protein have been demonstrated in the basal state (Kargman et al., 1996; Ferraz et al., 1997; Maricic et al., 1999; Jackson et al., 2000). Whether COX-3, a splice variant of

ABBREVIATIONS: PG, prostaglandin; NO, nitric oxide; l-NAME, Nω-monomethyl-l-arginine; COX, cyclooxygenase; DFU, 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(SII)-furanone; l-NAME, Nω-monomethyl-l-arginine methyl ester; d-NAME, Nω-nitro-d-arginine methyl ester; CGRP, calcitonin gene-related peptide.
COX-1 recently shown to occur in canine and human cerebral cortex and human heart (Chandrasekharan et al., 2002) is expressed in the stomach is not known so far. Most standard nonsteroidal anti-inflammatory drugs such as indomethacin inhibit COX-1 and COX-2 to a comparable extent (Meade et al., 1993). Inhibition of COX-1 was suggested to underlie the ulcerogenic activities and gastrointestinal side effects of nonselective nonsteroidal anti-inflammatory drugs, whereas COX-2 was considered to mediate proinflammatory reactions without contributing to the resistance of the gastric mucosa against damaging agents. This concept was strengthened by studies demonstrating that selective COX-2 inhibitors are not ulcerogenic in experimental animals (Masferrer et al., 1994) and cause less gastrointestinal side effects than standard nonsteroidal anti-inflammatory drugs in humans (Hawkey, 1999).

Recently, however, it was shown that in normal rats neither selective inhibition of COX-1 nor of COX-2 injures the gastric mucosa. Gastric lesions only developed when both COX isoenzymes were simultaneously inhibited (Wallace et al., 2000; Gretzer et al., 2001; Tanaka et al., 2001), implicating a role for COX-2 in gastric mucosal defense. This view is supported by observations that COX-2 represents a protective factor involved in gastric mucosal resistance in the face of pending injury. Thus, in the rat stomach, COX-2 inhibitors abolished the protection induced by the mild irritant 20% ethanol against a subsequent challenge with 70% ethanol (Gretzer et al., 1998), partially antagonized the protection caused by intragastric perfusion with peptone against ethanol-induced injury (Ehrlich et al., 1998), and markedly aggravated gastric damage induced by ischemia-reperfusion (Marcic et al., 1999). Furthermore, nonselective COX inhibitors and selective COX-2 inhibitors delayed the healing of chronic gastric ulcers in mice (Mizuno et al., 1997) and rats (Schmassmann et al., 1998) to the same extent.

The present study investigates whether the interaction of prostaglandins with NO and afferent neurons in the modulation of gastric mucosal integrity involves COX-1 and/or COX-2. To answer this question, we evaluated the effects of the selective COX-1 inhibitor 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylypyrazole (SC-560) (Smith et al., 1998), the selective COX-2 inhibitors N-[2-(cyclohexyloxy)-4-nitrofenyl]-methyleneasulfonamide (NS-398) (Futaki et al., 1994) and 5,5-dimethyl-3-(3fluorophenyl)-4-(4-methylsulfonyl)phenyl-2(5H)-furanone (DFU) (Riendeau et al., 1996) as well as the glucocorticoid dexamethasone, which inhibits the up-regulation of gastric COX-2 (Gretzer et al., 2001) on gastric mucosal integrity. The studies were performed in rats treated with the nonselective inhibitor of constitutive and inducible NO synthase N°-monomethyl-L-arginine methyl ester (L-NAME) (Rees et al., 1990) or the selective inhibitor of inducible NO synthase N-(3-(aminomethyl)benzyl)acetamide (1400W) (Garvey et al., 1997) to suppress endogenous NO, or with capsaicin to ablate afferent nerves. Furthermore, we investigated whether phenacetin, which was reported to selectively inhibit COX-3 (Chandrasekharan et al., 2002), mimics the effects of COX-1 and COX-2 inhibitors in NO-depleted rat gastric mucosa.

**Materials and Methods**

**Drugs.** DFU was a generous gift from Dr. A. W. Ford-Hutchinson (Merek Frosst Canada, Montreal, Canada). NS-398 was purchased from Cayman Chemical (Ann Arbor, MI), 1400W from QbioGene-Alexis (Grüningen, Germany), and capsaicin from Fluka (Neu-Ulm, Germany). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

**Animals.** Male Wistar rats (180–220 g) were fasted overnight with free access to tap water. The studies reported in this article have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. All experimental protocols were approved by the Animal Care Committee of the Ruhr-University of Bochum.

**Gastric Mucosal Damage.** Rats were anesthetized (pentobarbital, 50 mg/kg i.p.), tracheotomized, and the stomachs exposed by a mid-line incision. After ligation of the esophagus and pylorus, 1 ml of HCl (300 mM) was injected into the gastric lumen through the forestomach. Forty-five minutes later, the stomach was excised and gastric mucosal damage assessed. Further rats were treated intragastrically with 1 ml of water instead of acid. In sham-operated rats, the abdomen was opened in anesthesia and the esophagus and pylorus manipulated without ligations.

Gross mucosal damage was assessed in a blinded manner by calculation of a lesion index using a 0 to 3 scoring system based on the number and length of the lesions as described previously (Stroff et al., 1996). The severity factor was defined according to the length of the lesions: 0, no lesions visible; 1, lesions <1 mm; II, lesions 2 to 4 mm; and III, lesions >4 mm. The lesion index was calculated as the total number of lesions multiplied by their respective severity factor. Normal rats do not show gastric lesions (lesion index 0), a lesion index below 10 represents minor damage, and a lesion index above 30 represents severe damage.

For histological study, a strip of the stomach wall parallel to the limiting ridge was processed using routine methods, stained with H&E, and examined under a light microscope in a randomized blinded manner. One full-length portion of tissue (approximately 2 mm in width) across the entire corpus was cut from each stomach (section length, 3.1–4.2 cm). Two grades of histological injury were assessed: grade 1, superficial damage confined to the surface epithelium; and grade 2, deep damage extending beyond the surface epithelium into the region of pits and glands, as described previously (Gretzer et al., 1998). For each stomach strip, the length of mucosal areas showing superficial and deep damage was determined and expressed as percentage of the total section length, which is the full-length portion of tissue across the entire corpus, studied.

**Treatment Regimens.** Groups of rats were treated with the selective COX-2 inhibitors DFU (0.02–2 mg/kg s.c.) or NS-398 (0.01–1 mg/kg s.c.), the selective COX-1 inhibitor SC-560 (0.05–5 mg/kg s.c.), phenacetin (400 mg/kg s.c.) 60 min, or dexamethasone (1 mg/kg s.c.) 120 min before intragastric instillation of 1 ml of 300 mM HCl. These rats received concurrent treatment with the nonselective inhibitor of constitutive and inducible NO synthase L-NAME (40 mg/kg i.v.) 10 min before acid challenge. Phenacetin was used as it was reported to inhibit COX-3 more potently than its metabolite acetaminophen (Chandrasekharan et al., 2002). Other groups of rats treated with DFU (2 mg/kg s.c.) or NS-398 (1 mg/kg s.c.) received i.v. injections of the inactive enantiomer N°-nitro-D-arginine methyl ester (D-NAME, 3 or 40 mg/kg) or the selective inhibitor of inducible NO synthase 1400W (0.3 mg/kg) 10 min before acid challenge. Further groups of acid-challenged rats received single treatments with L-NAME (40 mg/kg i.v.), 1400W (0.3 mg/kg i.v.), DFU (2 mg/kg s.c.), NS-398 (1 mg/kg s.c.), SC-560 (5 mg/kg s.c.), dexamethasone (1 mg/kg s.c.), or phenacetin (400 mg/kg s.c.) instead of the combined treatment. NS-398 and SC-560 were dissolved in absolute ethanol; DFU and phenacetin in methyl sulfoxide; and dexamethasone, L-NAME, and D-NAME in saline. Controls received the corresponding vehicle.
As the observations were identical in rats treated with the various vehicles and did not differ from those in rats without treatment, results in vehicle-treated control rats were combined.

Functional Ablation of Afferent Neurons. Rats were treated with s.c. injection of capsaicin (25 and 50 mg/kg on day 1, 50 mg/kg twice on day 2). Capsaicin dissolved in 10% ethanol, 10% Tween 80, and 80% saline (v/v). All injections were performed under ether anesthesia, and to counteract the respiratory impairment associated with the administration of capsaicin, the rats were pretreated i.p. with terbutaline (0.2 mg/kg), atropine (0.2 mg/kg), and theophylline (20 mg/kg) before capsaicin injection. Rats were used for experiments 14 days after this treatment. On the day before the experiments, inactivation of afferent neurons was ascertained by evaluating the reduction of the protective wiping movements in response to intragastric instillation of a 0.1-mg/ml solution of capsaicin.

Capsaicin-pretreated rats received s.c. DFU (0.02–2 mg/kg), NS-398 (0.01–1 mg/kg), dexamethasone (1 mg/kg), or SC-560 (5 and 10 mg/kg) following the time schedule outlined above. These rats did not receive concurrent treatment with L-NAME. No differences in mucosal integrity were observed in capsaicin-pretreated rats that received either intragastric instillation of HCl alone or treatment with the various vehicles before acid instillation.

Effect of Prostaglandin Supplementation. To examine whether the effects of COX inhibitors and dexamethasone are related to suppression of endogenous prostaglandins, rats with intact or denervated afferent neurons received concurrent treatment with 16,16-dimethyl-PGE_2 (dissolved in 70% ethanol). The prostaglandin analog (8 ng/kg) was injected s.c. 5 min before treatment with DFU, NS-398, dexamethasone, or SC-560 and immediately before intragastric acid instillation. Rats with intact afferent neurons were treated additionally with i.v. injections of L-NAME 10 min before acid instillation. In these experiments, the dose of L-NAME was reduced to 3 mg/kg because pilot experiments had shown that the damage-inducing effects of COX inhibitors and dexamethasone could not be reversed by the prostaglandin analog in rats treated simultaneously with L-NAME at the high dose of 40 mg/kg. Capsaicin-pretreated rats did not receive additional injections of L-NAME. A flow sheet of the experimental procedures used is shown in Fig. 1.

To ascertain that 16,16-dimethyl-PGE_2 at the dose used has no general gastroprotective activity, the prostaglandin analog (8 ng/kg) was injected s.c. 65 min and immediately before intragastric challenge with 70% ethanol, and mucosal damage was assessed 5 min later.

Statistical Analysis. All data are expressed as mean ± S.E.M. from at least four or more animals. Comparisons between groups were made using the Wilcoxon rank test for nonparametric data. A p value of <0.05 was considered significant.

Results

Effect of Suppression of COX Isoenzymes in Rats with Intact Afferent Neurons. Sham operation and intragastric instillation of 1 ml of water did not induce any mucosal damage over the 45-min study period. Similarly, mucosal injury was negligible after instillation of acid (1 ml of 300 mM HCl) with a lesion index of 3 ± 0.4, n = 6. In acid-challenged rats, s.c. administration of DFU (2 mg/kg), NS-398 (1 mg/kg), dexamethasone (1 mg/kg), or phenacetin (400 mg/kg) when given alone caused no or only minor damage to the mucosa (Fig. 2A). Similarly, i.v. injection of L-NAME (40 mg/kg) alone induced only slight mucosal injury (Fig. 2B), whereas 1400W (0.3 mg/kg) had no effect (lesion index 3 ± 0.7, n = 4). However, in acid-challenged rats treated with L-NAME (40 mg/kg i.v.), concurrent administration of DFU (0.02–2 mg/kg s.c.) or NS-398 (0.01–1 mg/kg s.c.) caused marked and dose-related damage to the gastric mucosa (Fig. 2B). Likewise, dexamethasone (1 mg/kg s.c.) induced severe mucosal injury in L-NAME-treated rats, whereas phenacetin (400 mg/kg s.c.) was without effect (Fig. 2B). In rats treated with the inactive enantiomer D-NAME (3 or 40 mg/kg i.v.), DFU (2 mg/kg s.c.) induced no or significantly less gastric injury compared with rats treated with the corresponding dose of L-NAME. Likewise, D-NAME (3 mg/kg) did not provoke damage after administration of NS-398 (1 mg/kg) (Fig. 3). Furthermore, administration of DFU (2 mg/kg s.c.) or NS-398 (1 mg/kg s.c.) caused only slight and no mucosal damage, respectively, in rats treated with the selective inhibitor of inducible NO synthetase 1400W (Fig. 3). In contrast to COX-2 inhibitors and dexamethasone, SC-560 (0.05–5 mg/kg s.c.) moderately damaged the acid-challenged mucosa in a dose-dependent manner, even without concurrent treatment with L-NAME. This damage was significantly aggravated after simultaneous administration of L-NAME (40 mg/kg i.v.) (Fig. 4).

Effect of Suppression of COX Isoenzymes in Rats with Afferent Nerve Denervation. In rats pretreated with capsaicin to induce afferent nerve denervation, the gastric mucosa did not show any visible damage after sham operation (data not shown) and minor damage after intragastric instillation of acid (Fig. 5). Treatment with DFU (0.02–2 mg/kg s.c.), NS-398 (0.01–1 mg/kg s.c.), dexamethasone (1 mg/kg s.c.), or SC-560 (5 and 10 mg/kg s.c.) induced severe mucosal injury. For DFU, NS-398, and SC-560, the dose dependence of the damage-inducing effect was established (Fig. 5).

Effect of Prostaglandin Supplementation. Administration of 16,16-dimethyl-PGE_2 (8 ng/kg s.c.) 65 min and immediately before intragastric instillation of 1 ml of 70% ethanol did not inhibit ethanol-induced mucosal injury [lesion index 41 ± 2 (n = 4) versus 40 ± 1 (n = 7)] in rats treated with vehicle before 70% ethanol, indicating that at this low dose the prostaglandin analog has no general gastroprotective activity. In L-NAME-
treated rats with intact afferent neurons, 16,16-dimethyl-PGE2 (8 ng/kg s.c.) injected 5 min before administration of DFU (2 mg/kg s.c.), NS-398 (1 mg/kg s.c.), dexamethasone (1 mg/kg s.c.), or SC-560 (5 mg/kg s.c.) and immediately before acid instillation fully reversed the damage-inducing effect of the COX inhibitors and dexamethasone (Fig. 6).

Likewise, in capsaicin-pretreated rats, 16,16-dimethyl-PGE2 (8 ng/kg s.c., same time schedule as described above) prevented the development of mucosal damage induced by DFU (2 mg/kg s.c.), NS-398 (1 mg/kg s.c.), dexamethasone (1 mg/kg s.c.), or SC-560 (10 mg/kg s.c.) (Fig. 7).

**Histological Injury.** Sham-operated rats did not show any histological injury of the gastric mucosa (data not shown). Figure 8 shows the occurrence of superficial and deep histological injury (expressed as percentage of section length studied) in the various treatment groups. In rats with intact afferent neurons, histological injury was negligible after acid instillation alone. Furthermore, histological injury was negligible or absent in acid-challenged rats 2 weeks before experiments. Denervated rats received s.c. injections of DFU, NS-398, or SC-560 s.c. 60 min or dexamethasone 120 min before intragastric instillation of 1 ml of 300 mM HCl and damage was assessed 45 min later. Values are mean ± S.E.M. of six rats per group. *p < 0.05; **p < 0.01; ***p < 0.001 versus the corresponding dose of SC-560 in the absence of L-NAME.

Fig. 2. Effects of s.c. administration of the COX-2 inhibitors DFU and NS-398, dexamethasone, or the COX-3 inhibitor phenacetin in the gastric mucosa of rats with intact afferent neurons. Controls (Co) received the vehicles. Rats were challenged with 1 ml of 300 mM HCl 60 min (DFU, NS-398, and phenacetin) or 120 min (dexamethasone) after drug administration, and gastric damage was assessed 45 min later. A, effects in acid-challenged rats without concurrent treatment with L-NAME. B, effects in rats with concurrent treatment with L-NAME (40 mg/kg i.v., 10 min before acid challenge). Values are mean ± S.E.M. of four to six rats per group. *p < 0.05; **p < 0.01; ***p < 0.001 versus the acid-control group.

Fig. 3. Lesion formation induced by DFU or NS-398 in rats with intact afferent neurons treated with L-NAME, D-NAME, or 1400W. DFU (2 mg/kg) or NS-398 (1 mg/kg) was injected s.c. 60 min before intragastric instillation of 1 ml of 300 mM HCl, and damage was assessed 45 min later. Rats received concurrent treatment with L-NAME, D-NAME, or 1400W i.v. 10 min before acid challenge. Values are mean ± S.E.M. of six to seven rats. *p < 0.05; **p < 0.01 versus the corresponding COX-2 inhibitor alone; ***p < 0.001 versus the corresponding L-NAME-treated group.

Fig. 4. Effect of SC-560 on the gastric mucosa of acid-challenged rats with or without concurrent treatment with L-NAME. SC-560 was injected s.c. 60 min before instillation of 1 ml of 300 mM HCl and damage was assessed 45 min later. Rats received concurrent i.v. treatment with either vehicle or L-NAME (40 mg/kg) 10 min before acid challenge. Values are mean ± S.E.M. of six rats per group. *, p < 0.05; ***, p < 0.001 versus the acid-control group. $, p < 0.05 versus the corresponding dose of SC-560 in the absence of L-NAME.

Fig. 5. Effects of DFU, NS-398, dexamethasone, and SC-560 in the gastric mucosa of rats with afferent nerve denervation. Rats were pre-treated with capsaicin to induce ablation of afferent neurons 2 weeks before experiments. Denervated rats received s.c. injections of DFU, NS-398, or SC-560 s.c. 60 min or dexamethasone 120 min before intragastric instillation of 1 ml of 300 mM HCl and damage was assessed 45 min later. Values are mean ± S.E.M. of six rats per group. ***, p < 0.001 versus the denervated acid-control group.
High levels of COX-1 mRNA occur in the gastric mucosa of normal (Kargman et al., 1996; Ferraz et al., 1997) or sham-operated rats and after intragastric instillation of saline, whereas levels of COX-2 mRNA are low (Gretzer et al., 2001). Instillation of acid (300 mM HCl) resulted in up-regulation of mucosal COX-2 mRNA without change in the expression of COX-1 (Gretzer et al., 2001). Acid-induced expression of COX-2 mRNA in the rat stomach was also reported by Sawaoka et al. (1997). These authors, however, used a higher concentration of acid (600 mM HCl) that caused severe macroscopic mucosal injury and widespread histological changes. Sawaoka et al. (1997) concluded that the up-regulation of gastric COX-2 is the consequence of the injury after instillation of concentrated acid. In our experiments using a lower concentration of acid, no macroscopic and histological damage was found, indicating that acid can induce COX-2 expression in the absence of mucosal damage (Gretzer et al., 2001). Our experiments also showed that dexamethasone prevents the gastric up-regulation of COX-2 associated with acid instillation, whereas levels of COX-1 mRNA were not affected.

The present study confirms that in rats with intact afferent neurons the macroscopic and histological appearance of the gastric mucosa after instillation of 300 mM HCl is comparable with that in sham-operated rats or rats treated with

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<th>TABLE 1 Effect of inhibition of afferent nerve function, NO, COX-1, or COX-2 alone or in combination on rat gastric mucosal integrity</th>
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+, intact; –, inhibited.
intragastric water. After administration of L-NAME, DFU, NS-398, or dexamethasone alone, mucosal damage remained negligible. However, when L-NAME-treated rats received concurrent treatment with the COX-2 inhibitors DFU or NS-398 or dexamethasone before acid instillation, severe and dose-related mucosal injury developed. The specific nature of this action of L-NAME was demonstrated by the finding that DFU and NS-398 induced significantly less gastric damage in rats treated with the inactive enantiomer d-NAME compared with L-NAME-treated rats.

NO involved in the regulation of gastric mucosal integrity under the experimental conditions used in the current study is generated predominantly via the constitutive NO synthase pathway. This is suggested from the finding that the ulcerogenic potency of DFU and NS-398 after treatment with 1400W, a highly selective inhibitor of inducible NO synthase (Garvey et al., 1997), is significantly lower or absent compared with that after treatment with L-NAME, a dual inhibitor of constitutive and inducible NO synthase (Rees et al., 1990). Increasing the dose of 1400W to 1 mg/kg or prolonging the period between administration of COX-2 inhibitors and injection of 1400W to 80 min did not increase the ulcerogenic activity of the COX-2 inhibitors (data not shown). The dose of 1400W (0.3 mg/kg) used in the present study was previously shown to abolish the lack of ulcerogenicity of the nonsteroidal anti-inflammatory drug amtolmetin guacyl in the rat stomach (Peskar et al., 2002). The low gastrototoxicity of amtolmetin guacyl has been attributed to up-regulation of the inducible NO synthase (Coruzzi et al., 2002).

In normal rats, selective inhibition of COX-1 or COX-2 did not injure the gastric mucosa and damage only developed after simultaneous suppression of both isoenzymes (Wallace et al., 2000; Gretzer et al., 2001; Tanaka et al., 2001). Although after intragastric instillation of acid COX-2 inhibitors were still devoid of ulcerogenic properties, mucosal damage occurred after administration of SC-560 (Gretzer et al., 2001). Obviously, in the normal stomach, mucosal integrity is preserved when either COX-1 or COX-2 is functioning. In contrast, in the face of pending injury, COX-2 alone is not sufficient to ensure the maintenance of mucosal viability. This observation was confirmed in the present study where SC-560 caused dose-dependent mucosal injury in the acid-challenged stomach even without suppression of NO synthase. SC-560-induced damage was, however, significantly aggravated after cotreatment with L-NAME. Together, these findings show that the endogenous NO system limits or even prevents the damaging effects of inhibitors of both COX-1 and COX-2. This is in line with observations that NO-releasing nonsteroidal anti-inflammatory drugs cause markedly reduced damage to the gastric mucosa (Wallace et al., 1994).

Recently, a third COX isoenzyme, COX-3, was described in canine and human cerebral cortex and human heart (Chandrasekharan et al., 2002). Whether this splice variant of COX-1 also occurs in gastric tissues has not been investigated. Chandrasekharan et al. (2002) found that various nonsteroidal anti-inflammatory drugs block the activity of COX-3 with phenacetin being the most selective inhibitor. Treatment of rats with phenacetin (400 mg/kg s.c.) did not damage the gastric mucosa in L-NAME-treated and acid-challenged rats. Increasing the dose of phenacetin to 800 mg/kg had not more effect (data not shown). Phenacetin administered i.p. at 400 mg/kg significantly reduced the intensity of hyperalgesia assessed in a modified Randall-Selitto test, indicating in vivo activity of the drug at this dose (Ferreira et al., 1978). The lack of effect of phenacetin in our experiments does not support a role for COX-3 in gastric mucosal resistance, although further studies are needed to elucidate a possible contribution of this COX isoform in various gastric functions.

In rats treated with capsaicin to deplete neuropeptides, the presence of intragastric acid did not induce any macroscopic or histological damage. After afferent nerve denervation, administration of DFU, NS-398, dexamethasone, or SC-560 caused severe gastric lesions even without concurrent treatment with L-NAME. Dose dependence of the injurious effects was demonstrated for the COX-1 inhibitor and the COX-2 inhibitors.

Neuropeptide-containing afferent neurons are present in abundance in the gastric mucosa (Sternini et al., 1987). Activation of afferent neurons by a low dose of capsaicin exerts protection against a variety of ulcerogens (Holzer, 1998). This protection is mainly mediated by release of the neuropeptide calcitonin gene-related peptide (CGRP) (Lambrecht et al., 1993). The protective effects of both afferent nerve stimulation and CGRP are abolished by pretreatment with L-NAME. From these findings, we have proposed that afferent nerve stimulation promotes the release of CGRP, which activates formation of NO. NO then represents the final mediator of protection (Lambrecht et al., 1993). High activity of constitutive NO synthase has been measured in rat gastric mucous-epithelial cells (Brown et al., 1992). Vascular endothelial cells (Moncada et al., 1991) and gastrointestinal smooth muscle cells (Grider et al., 1992) have the capacity to synthesize NO. CGRP released from nerve terminals may stimulate NO formation in gastric mucous-epithelial, endothelial, or smooth muscle cells. In addition, NO synthase has been immunolocalized in neurons in the myenteric plexus of the gastrointestinal tract (Bredt et al., 1990). Furthermore, NO is released upon electrical stimulation of nonadrenergic noncholinergic nerves in the rat stomach fundus (Boekxstaens et al., 1991). Whether neuronal derived NO contributes to protective effects has not been established. The sequence outlined above can explain why both depletion of neuropeptides and blockade of NO synthase interfere with the maintenance of gastric mucosal integrity when prostaglandin formation is reduced.

SC-560 potently inhibited formation of gastric 6-keto-PGF1α, and PGE2 and platelet thromboxane B2 as expected for a COX-1 inhibitor (Gretzer et al., 2001). The COX-2 inhibitors DFU and NS-398 at doses comparable with those used in the present study did not inhibit platelet thromboxane B2 formation (Gretzer et al., 1998; Maricic et al., 1999; Gretzer et al., 2001), indicating that the effects observed with these compounds are not accounted for by lack of selectivity. We and others (Gretzer et al., 1998, 2001; Maricic et al., 1999; Wallace et al., 2000) could not demonstrate that in rats COX-2 inhibitors reduce prostaglandin formation in nonulcerated gastric mucosa. This phenomenon may be due to the fact that COX-2-derived prostaglandins represent only a small portion of the total gastric mucosal prostaglandin pool, which renders isolated assessment of changes of COX-2 activity difficult. NS-398 and DFU, however, reduced PGE2 levels in rat inflammatory tissue, indicating potent COX-2 inhibitory activity (Gretzer et al., 1998, 2001).
that the effects of DFU and NS-398 observed in l-NAME-treated and denervated rats are due to suppression of prostaglandin formation is supported by the finding that supplementation with the prostaglandin analog 16,16-dimethyl-PGE$_2$ antagonized the injurious effects of the COX-2 inhibitors. Administration of 16,16-dimethyl-PGE$_2$ also counteracted the damage induced by dexamethasone and SC-560 in l-NAME-treated and denervated rats. The dose of 16,16-dimethyl-PGE$_2$ used did not protect against 70% ethanol-induced gastric injury. This suggests that the protective effect of the prostaglandin analog shown in the present experiments results from replacement of suppressed endogenous prostaglandins and not from an unspecific protective activity.

Our results support the concept suggested by Whittle et al. (1990) that endogenous NO, prostaglandins and sensory neuropeptides act in concert in the regulation of gastric mucosal integrity. The finding that the selective COX-2 inhibitors NS-398 and DFU as well as the selective COX-1 inhibitor SC-560 induce gastric damage in rats with suppressed NO bioynthesis or depleted neuropeptides suggests that the prostaglandins necessary to maintain mucosal integrity despite depression of other members of the gastric mucosal defense system can be derived either from COX-1 or COX-2. The findings further support the concept (Wallace et al., 2000; Greter et al., 2001) that both COX-1 and COX-2 have important functions in the resistance of the gastric mucosa to damaging agents but do not suggest a protective role for COX-3.

References


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