JPET Fast Forward. Published on September 15, 2003 as DOI:10.1124/jpet.103.054973

JPET#54973

Cyclooxygenase Isozymes Involved in Adaptive Functional Responses in Rat Stomach Following Barrier Disruption

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Running title: COX Isozymes and Gastric Barrier Disruption

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Document statistics: text ---------- 17 pages (page 3-page 19)
figures ---------- 8 figs
references ---------- 37 refs. (page 20-page 24)

Number of words: abstract ---------- 242
introduction ----------409
discussion ---------- 1331

Recommended section: Gastrointestinal, Hepatic, Pulmonary, & Renal

Abbreviations:

nonsteroidal antiinflammatory drug ---------- NSAID
taurocholate ---------- --TC
prostaglandin ---------- PG
cyclooxygenase-1 ---------- COX-1
cyclooxygenase-2 ---------- COX-2
gastric mucosal blood flow ---------- GMBF
potential difference ---------- PD
reversed transcriptional polymerase reaction ---------- RT-PCR

Abstract

We investigated the preferential role of cyclooxygenase (COX) isozymes in various functional changes of the rat stomach following exposure to taurocholate (TC) as a mild irritant. Under urethane anesthesia, a rat stomach mounted in an ex-vivo chamber was perfused with saline or acid (50 mM HCl), and transmucosal potential difference (PD), mucosal blood flow (GMBF) and acid secretion were measured before and after exposure of the stomach to 20mM TC for 30 min. Indomethacin, SC-560 (a selective COX-1 inhibitor) or rofecoxib (a selective COX-2 inhibitor) was given intraduodenally 30 min before the TC treatment. Mucosal application of TC caused a marked reduction in PD, followed by a decrease of acid secretion and an increase of GMBF. Prior administration of indomethacin did not affect the reduction in PD but significantly mitigated the two other responses induced by TC, resulting in a delay in the recovery in PD. These effects were mimicked by SC-560 but not rofecoxib, although neither of these drugs had any effect on the reduction in PD. Perfusion of TC-treated stomachs with 50 mM HCl caused only minimal damage, yet this treatment produced gross lesions in the presence of indomethacin or SC-560. Mucosal exposure to TC increased PGE_2 production, but the response was inhibited by both indomethacin and SC-560 but not rofecoxib. These results suggested that COX-1 but not COX-2 is a key enzyme for regulating the functional alterations of the stomach and for maintaining the mucosal integrity after barrier disruption.

In the gastrointestinal tract tissues, prostaglandins (PGs) are involved in a variety of physiological processes, including gastric secretion, production of mucus, mucosal blood flow and maintenance of mucosal integrity (Robert & Ruwart, 1982). The key enzyme in the pathway for PG synthesis, cyclooxygenase (COX), exists as two isozymes referred to as COX-1 and COX-2. COX-1, constitutively expressed in normal gastric mucosa, has been proposed to generate PGs involved in the maintenance of essential physiological functions (Simmons et al., 1991; O'Neill & Ford-Hutchinson, 1993) while COX-2, characterized by a rapid inducibility in response to various proinflammatory stimuli, has been thought to be responsible for pathological PG production at inflammatory sites (O'Banion et al., 1991; Xie et al., 1992). Studies using selective COX-2 inhibitors showed that the gastric ulcerogenic property of nonsteroidal antiinflammatory drugs (NSAIDs) is brought about by inhibition of COX-1 but not COX-2 (Whittle, 1983; Futaki et al., 1993). However, it has been recently shown that inhibition of both COX-1 and COX-2 is required for NSAID-induced gastric injury, suggesting a role for COX-2 as well as COX-1 in maintaining the integrity of the stomach (Wallace et al., 2000; Tanaka et al., 2001).

It is known that the application of mild irritants to the stomach causes an increase in gastric mucosal blood flow (GMBF) as well as a decrease in acid secretion (Whittle, 1983; Takeuchi et al., 1986; 1993). These agents damage the surface epithelium of the gastric mucosa, resulting in acid back-diffusion, yet they rarely cause macroscopically visible damage and actually protect the stomach against necrotizing agents (Holzer et al., 1991; Takeuchi et al., 1993). Such hyperemic responses subside in the presence of NSAIDs, suggesting a role for endogenous PGs in this phenomenon (Whittle, 1983; Nobuhara et al., 1984; Takeuchi et al., 1986; 1987). We previously reported, using a selective COX-2 inhibitor, that endogenous PGs produced by COX-1 play an important role in maintaining the gastric hyperemic

response and mucosal integrity following barrier disruption by taurocholate Na (TC)(Hirata et al., 1997), yet the relative contribution of the COX-1 and COX-2 isozymes to the maintenance of other functional responses such as acid secretion in the stomach following barrier disruption is not entirely clear.

In the present study, we examined the effects of selective COX-1 and COX-2 inhibitors on changes in various functions of the rat stomach following exposure to TC and investigated the role of COX isozymes in functional responses of the stomach under adverse conditions, ie., after barrier disruption.

Materials and Methods

Animals

Male Sprague-Dawley rats, weighing 200~230 g (Charles River, Shizuoka, Japan), were used in all experiments. The animals were kept in individual cages with raised mesh bottoms and deprived of food but allowed free access to tap water for 18 hr prior to the experiments. Studies were carried out using 4~6 rats per group. All experimental procedures described here were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Experimental Protocol

The experiments were performed in four groups of rats; each group was pretreated with saline, indomethacin (a nonselective COX-1 and COX-2 inhibitor), SC-560 (a selective COX-1 inhibitor)(Smith et al., 1998) and rofecoxib (a selective COX-2 inhibitor)(Chan et al., 1995), respectively. In these groups of rats, the effects of mucosal application of 20 mM TC plus 50 mM HCl on gastric transmucosal potential difference (PD), gastric mucosal blood flow (GMBF), luminal acid loss (acid back-diffusion), acid secretion and PGE_2 production were examined under urethane anesthesia. Various COX inhibitors were given intraduodenally at a dose of 5 mg/kg, 30 min before the TC treatment. In some rats, the expression of mRNA for COX-1 and COX-2 was examined using reverse transcription-polymerase chain reaction (RT-PCR) in the stomach after exposure to TC. In a separate study, we also examined the effects of these COX inhibitors on the production of PGE_2 in carrageenan-induced paw edema in rats.

Determination of PD, GMBF, and Acid Back-Diffusion

Animals were anaesthetized with urethane (1.25 g/kg, intraperitoneally), and the trachea was cannulated to ensure a patent airway. Acid secretion was

completely inhibited by pretreatment with omeprazole (60 mg / kg, intraperitoneally). Simultaneous measurement of PD, GMBF, and acid back-diffusion was performed in a chambered stomach as previously described (Matsumoto et al., 1992; Takeuchi et al., 1994). Briefly, the abdomen was incised, and the stomach was exposed and mounted on an *ex-vivo* chamber (area exposed, 3.14 cm^2). At the beginning of each experiment, the mucosa was rinsed several times with a solution of 50 mM HCl plus 100 mM NaCl. When the gastric exudate became clear, 2 ml of the acid solution was instilled in the chamber, and 15 min later the gastric contents were recovered from the chamber. This procedure was repeated every 15 min, two times before and 6 times after exposure of the mucosa to 20 mM TC plus 50 mM HCl for 30 min. PD was determined using two agar bridges, one positioned in the chamber and the other in the abdominal cavity. GMBF was measured with a laser Doppler flowmeter (Advance Model ALF 21, Tokyo, Japan), placing the probe gently on the corpus mucosa using a balancer, and changes in GMBF were continuously monitored on a two-channel recorder (U-228, Tokai-Irika, Tokyo, Japan) simultaneously with those of PD. Acid back-diffusion (luminal acid loss) was determined from analyses of the collected acid solution. Each sample was analyzed for volume and acid concentration, which was determined by automatic titration of an aliquot with 0.1 N NaOH to pH 7.0 (Autoburette, Comtite-7, Hiranuma, Tokyo, Japan). The amount of acid back-diffusion was calculated as the difference between the product of the final volume and concentration and the product of the initial volume and concentration. Positive values indicate that the net flux of H^+ was from the mucosa to the lumen, and the results were expressed as microequivalents per 15 min. At the end of each experiment, ie., 90 min after exposure to TC, the mucosa was examined for hemorrhagic damage under a dissecting microscope with a square grid (x10). The area (mm^2) of each lesion was measured, summed per stomach and used as a lesion

score. The person measuring the lesions did not know the groups to which test drugs were given. Tissue samples were then immersed into 10% formalin for histological observation, processed for routine light microscopy, sectioned at 5 μm, and stained with hematoxylin and eosin.

Determination of Acid Secretion

The experiment was performed using the same chamber system described above, except that the animals were not pretreated with omeprazole (Takeuchi et al., 1994). The mucosa mounted on a chamber was superfused at a flow rate of 1 ml/min with saline that was suffused with 100% O_2, and kept in a reservoir. Acid secretion was measured by introducing an automatic titrator (Hiranuma Comtite-8, Tokyo, Japan) into the perfusion system, and the titration was performed at luminal pH 7.0 using the pH-stat method and by adding 50 mM NaOH to the reservoir, in which both entry and exit tubes were positioned. After basal acid secretion had stabilized, the mucosa was exposed to 20 mM TC for 30 min. After application of TC, the mucosa was rinsed with saline, another 2 ml of saline was instilled and the perfusion was resumed.

Determination of Prostaglandin E_2

Gastric mucosa: Mucosal PGE_2 levels were measured in the stomach with or without TC treatment. In the former, animals without anesthesia were given indomethacin, SC-560 or rofecoxib orally, and then killed 3 hr later under deep ether anesthesia. In the latter, the chambered stomach of urethane-anesthetized animals was exposed to 20 mM TC plus 50 mM HCl for 30 min, and the mucosa was excised 60 min after the exposure. Indomethacin, SC-560 or rofecoxib was given intraduodenally 30 min before the TC treatment. In both studies, the corpus mucosa was isolated, weighed, and placed in a tube containing 100% ethanol plus 0.1 M indomethacin (Futaki et al., 1994). The samples were then minced with scissors,

homogenized and centrifuged at 12000 r.p.m. for 10 min at 4℃. The supernatant of each sample was used for determination of PGE_2 by EIA using a PGE_2- kit (Cayman Chemical Co., Ann Arbor, MI).

Carrageenan-airpouch model: Effects of various COX inhibitors on PGE_2 content were examined in the exudates of a carrageenan-airpouch model. An airpouch was induced as described in detail previousy (Edwards et al., 1981; Seibert et al., 1994, Barnett et al., 2000). In brief, 20 ml of air was injected subcutaneously on the back of the rat on the first day. Two days later, another 10 ml of air was injected at the same site. On the fifth day after the first injection, a further 10 ml of air was injected into the pouch. Twenty-four hours later, carrageenan (2 ml of a 1% w/v solution in saline) was injected into the airpouch. All of the injections were performed under light ether anesthesia. Six hours after the carrageenan injection, the rats were anesthetized with ether, and the pouch was carefully opened by making a small incision. Then, the exudate was collected and transferred to a tube. An aliquot of the exudates was frozen on dry ice and stored at -20℃ for subsequent measurements of PGE_2 concentration as described above. Indomethacin, SC-560 or rofecoxib was given orally 1 hr before the last injection of carrageenan into the airpouch.

Analyses of COX-1 and COX-2 mRNAs by RT-PCR

The stomachs were exposed to 20 mM TC plus 50 mM HCl for 30 min and quickly removed from the chamber at 30 min or 90 min after the exposure. Each tissue was then frozen in liquid nitrogen and stored at -80℃ until use. The tissue samples were pooled from 2~3 rats for extraction of total RNA, which was prepared by a single-step acid phenol-chloroform extraction procedure by use of TRIZOLE (GIBCO BRL, Gaithersburg, MD). Total RNA primed by random hexadeoxy ribonucleotide was reverse-transcribed with the SUPERSCRIPT preamplification

system (GIBCO BRL). The sequences of sense and antisense primers for rat COX-1 and COX-2 as well as glyceraldehyde-3-phosphate dehydrogenase (G3PDH) are referred to the previous papers (Bredt et al., 1991; Feng et al., 1993; Iso et al., 1995). An aliquot of the RT reaction product served as a template in 35 cycles of PCR with 1 min of denaturation at 94℃, 0.5 min of annealing at 58℃ and 1 min of extension at 72℃ on a thermal cycler. A portion of the PCR mixture was electrophoresed in 1.8% agarose gel in TAE buffer (Tris buffer 40 mM, EDTA 2 mM and acetic acid 20 mM; pH 8.1), and the gel was stained with ethidium bromide and photographed.

Preparation of Drugs

Drugs used in this study were urethane (Tokyo Kasei, Tokyo, Japan), taurocholate Na (Difco Lab., Detroit, MI,), indomethacin, (Sigma Chemicals, St. Louis, Mo), SC-560 (Cayman Chemical, Ann Arbor, MI), rofecoxib (synthesized by our group), carrageenan (Nacalai tesque, Kyoto, Japan) and omeprazole (Astra Zeneca, Möndal, Sweden). All COX inhibitors were suspended in a hydroxy propyl cellulose (HPC) solution (Wako, Osaka, Japan). Omeprazole was suspended in a 0.5% carboxymethylcellulose solution. Other agents were dissolved in saline. Each agent was prepared immediately before use and administered i.p. or i.d. in a volume of 0.5 ml per 100 g body weight, or applied topically to the chamber in a volume of 2 ml.

Statistics

Data are presented as the mean±SE from 4~6 rats per group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test, and values of $p<0.05$ were regarded as significant.

JPET Fast Forward. Published on September 15, 2003 as DOI: 10.1124/jpet.103.054973
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Results

Effects of Various COX Inhibitors on PGE_2 Production in the Gastric Mucosa and the Carrageenan-Induced Airpouch

Gastric mucosa: Oral administration of indomethacin at 5 mg/kg caused a marked decrease in the mucosal PGE_2 content by about 80% at 1 hr after administration in the stomach, from 47.0±10.5 to 7.3±1.2 ng/g tissue **(Figure 1A)**. Likewise, SC-560 at 5 mg/kg also caused a significant decrease in PGE_2 content. However, rofecoxib at 5 mg/kg had no effect on mucosal PGE_2 content in the stomach.

Airpouch: Indomethacin given orally at 5 mg/kg significantly reduced exudate PGE_2 content in the carrageenan-induced airpouch **(Figure 1B)**. Rofecoxib at 5 mg/kg also significantly decreased the exudate PGE_2 content as effectively as indomethacin, while SC-560 at 5 mg/kg did not significantly affect the production of PGE_2 in the carrageenan-induced airpouch model.

Since SC-560 and rofecoxib at 5 mg/kg significantly inhibited the production of PGE_2 in the gastric mucosa and the airpouch exudate, respectively, these drugs at the dose were used in the subsequent studies as a selective COX-1 or COX-2 inhibitor.

Effects of Various COX Inhibitors on Gastric Functional Responses Induced by TC

PD response: Under chambered conditions in the presence of omeprazole (inhibition of acid secretion) and exogenous acid (50 mM HCl plus 100 mM NaCl), a rat stomach generated a PD of –59~68 mV (mucosa negative) and maintained a relatively constant GMBF during a 2-hr experimental period. In control rats, the

mucosal application of 20 mM TC plus 50 mM HCl for 30 min caused a marked reduction of PD from -63.3±2.8 mV to -30.8±2.6 mV, but after exposure the reduced PD was gradually normalized toward basal values, the degree of recovery being 49.6±4.6% at 90 min after treatment **(Figures 2A and 2B)**. Pretreatment of the animals with indomethacin, a nonselective COX-1 and COX-2 inhibitor, did not affect the reduction in PD caused by TC but significantly delayed the recovery of PD after exposure to TC, the degree of recovery being 12.4±7.2% at 90 min post-treatment. Likewise, SC-560, a selective COX-1 inhibitor, also had no effect on PD reduction but significantly caused a delay in PD recovery, similar to indomethacin. By contrast, a selective COX-2 inhibitor rofecoxib did not have any effect on such PD responses after exposure to TC; the degree of PD recovery at 90 min post-treatment was 47.2±10.9%, which was not statistically significant when compared to control rats.

GMBF response: The GMBF significantly increased during exposure to 20 mM TC (plus 50 mM HCl), reaching a peak increase of 118.4±27.4%, and remained significantly elevated for 30 min even after removal of TC from the chamber **(Figure 3)**. Even at 60 min after treatment, the GMBF showed a significant increase (~35%) as compared to pre-exposure values. This hyperemic response caused by TC was almost totally attenuated in rats pretreated with either indomethacin or SC-560, and in these animals the GMBF remained in similar ranges before and after exposure to TC, the peak increase being only ~20% above basal levels. By contrast, rofecoxib had no effect on the gastric hyperemic response induced by TC, and the peak increase in GMBF was 84.3±17.3%, which was not significantly different from that observed in control animals.

Acid back-diffusion: When the stomach was exposed to 50 mM HCl in the absence of acid secretion induced by omeprazole, a small but significant loss of luminal H^+ was consistently observed in control rats under normal conditions; acid

loss ($\triangle H^+$) was less than 12 μEq/15 min. Following the mucosal application of 20 mM TC, the loss of H^+ was significantly increased, reaching a maximal value (23.8±1.8 μEq/15 min) immediately after the exposure, then gradually decreasing to pre-exposure levels 120 min later **(Figure 4)**. Pretreatment with neither indomethacin, SC-560 nor rofecoxib had a significant effect on the increased mucosal permeability to H^+ in response to TC; the magnitude of $\triangle H^+$ observed immediately after TC treatment was 24.7±2.0, 23.8±2.1 and 19.8.5±2.9 μEq/15 min, respectively.

Acid secretion: The chambered stomach secreted acid to keep the luminal pH at around 3.5 under urethane-anesthetized conditions. Mucosal application of 20 mM TC for 30 min reduced acid secretion by about 60% from 10.1±1.8 μEq/10 min to 3.8±0.4 μEq/10 min (**Figure 5)**. This decrease in acid secretion induced by TC was significantly attenuated by prior administration of SC-560 as well as indomethacin, while rofecoxib had no effect on the reduced acid response to TC. In the group treated with indomethacin or SC-560, the acid secretion was also decreased by 20~30% following the mucosal exposure to TC, yet the changes were not statistically significant when compared to the pre-exposure values. In the animals treated with rofecoxib, however, the acid secretion markedly decreased in response to TC, the degree of change being similar to that observed in the contro group.

Effects of Various COX Inhibitors on Mucosal Ulcerogenic Response Induced by TC

Mucosal application of 20 mM TC plus 50 mM HCl produced a few hemorrhagic lesions in the gastric mucosa of control rats, the lesion score being 3.6±0.4 mm^2 **(Figure 6)**. When animals were pretreated with indomethacin, the lesions induced by TC plus HCl were significantly aggravated; the lesion score was 19.2±6.1 mm^2, which was about 5 times greater than that in the control animals.

Histologically, the stomachs of control rats only exhibited widespread exfoliation of epithelial cells without damage beyond the basement membrane, whereas in those pretreated with indomethacin the damage was deep into the mucosa with hemorrhagic changes (not shown). Likewise, SC-560 also significantly aggravated the mucosal ulcerogenic response to TC, the lesion score being 16.8±2.8 mm^2. In contrast, rofecoxib did not significantly affect the development of gastric lesions in response to TC, the lesion score being 5.1±2.6 mm^2.

Effects of Various COX Inhibitors on Changes in Mucosal PGE_2 Content Induced by TC

Mucosal exposure to 20 mM TC plus 50 mM HCl for 30 min stimulated PG biosynthesis to increase PGE_2 content to about 10-fold the basal value (from 38.6±4.6 to 685.2±201.4 ng/g tissue)**(Fgure 7)**. The PGE_2 biosynthetic response induced by TC was totally blocked by prior i.d. administration of indomethacin. The increase in mucosal PGE_2 was also significantly prevented by pretreatment with SC-560, the inhibition being 82.3%. In contrast, rofecoxib had no effect on the increased PG production after TC treatment.

Expression of COX-1- and COX-2-mRNAs in the Stomach

Under normal conditions, only COX-1 gene expression was detected in the gastric mucosa and remained unchanged after exposure to 20 mM TC plus 50 mM HCl for 30 min **(Figure 8)**. On the other hand, the gene expression of COX-2 was negligible in the rat stomach, with or without TC treatment, when determined even at 90 min after the exposure.

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Discussion

The present study confirmed a mediator role for endogenous PGs in the gastric functional responses in the rat stomach following barrier disruption by TC, and demonstrated that the gastric mucosa under such conditions was ulcerated when the animals were pretreated with indomethacin as well as SC-560 but not rofecoxib. These results suggest that COX-1 but not COX-2 is a key enzyme responsible for these functional responses observed acutely after barrier disruption and plays a role in protecting the gastric mucosa against acid injury.

First, we tested the activity of SC-560 or rofecoxib as a selective COX inhibitor. COX-1 is constitutively expressed in normal gastric mucosa and considered to generate PGs involved in the maintenance of essential physiological functions (Simmons et al., 1991; Whittle, 1983; Futaki et al., 1993). Mucosal PGE_2 content in the normal stomach was significantly decreased by SC-560 but not rofecoxib. By contrast, rofecoxib but not SC-560 suppressed PGE_2 production in a carrageenan-induced airpouch model. Since intrapleural injection of carrageenan produces an increase of PGE_2 production and induction of de novo synthesis of COX-2 in pleural exudate cells (Masferrer et al., 1994; Nakatsugi et al., 1996), there is no doubt that this rofecoxib action is due to suppression of COX-2 activity. Thus, the present results confirmed that SC-560 and rofecoxib at the dose used, ie., 5 mg/kg, selectively inhibits COX-1 and COX-2 activity, respectively.

It is known that the stomach responds to mucosal damaging agents by altering various functions such as mucosal blood flow (Nobuhara et al., 1984; Takeuchi et al., 1986; 1993). Although the role of PGs may vary with different types of mucosal irritants used to break the gastric mucosal barrier in the presence of luminal acid, this process is essentially mediated by endogenous PGs when the

barrier is disrupted by TC (Hirata et al., 1997; Nobuhara et al., 1984; Takeuchi et al., 1986). TC also damages surface epithelial cells and increases PG production in the gastric mucosa (Takeuchi et al., 1987; 1993), yet it remains unclear whether this PG response is mediated by the enzymatic activity of COX-1 or COX-2.

In the present study, we observed that the mucosal application of TC caused a reduction in PD followed by an increase in acid back-diffusion and GMBF, without extension to gross damage, consistent with previous findings of others and ourselves (Takeuchi et al., 1987; 1993). This treatment also enhanced PG biosynthesis in the stomach, inasmuch as the mucosal PGE_2 content increased to 10-fold the basal value after exposure to TC. Whittle et al (1983) showed that gastric hyperemia following acid back-diffusion caused by TC is attenuated by indomethacin, suggesting the involvement of endogenous PGs in this phenomenon. As expected, we observed in this study that SC-560, a selective COX-1 inhibitor, as well as indomethacin attenuated the hyperemic response to TC, without affecting PD reduction and acid back-diffusion, resulting in aggravation of gastric lesions following TC treatment. In contrast, the selective COX-2 inhibitor rofecoxib had no effect on the gastric hyperemic response following barrier disruption by TC and did not cause any gross damage in the stomach. Furthermore, SC-560 but not rofecoxib significantly decreased the increase in the mucosal PGE_2 content induced by TC, suggesting that the enhanced PG production following barrier disruption is associated with COX-1 activity. These findings also indicate that the gastric hyperemic response following acid back-diffusion is mediated by PGs and is dependent on COX-1 enzymatic activity. It is known that the gastric mucosa responds to damaging agents by decreasing acid secretion with a concomitant increase of mucosal blood flow (Nobuhara et al., 1984; Takeuchi et al., 1986; 1987). In the present study, we also confirmed that mucosal exposure to 20 mM TC caused surface cell damage as

represented by a reduction in PD, followed by a decrease of acid secretion. The reduced acid response in the stomach exposed to TC was significantly reverted by both indomethacin and SC-560, consistent with the present finding that endogenous PGs produced by COX-1 are involved in the GMBF response in the stomach following barrier disruption. As expected, rofecoxib did not have any effect on the reduced acid response in the stomach after exposure to TC, again confirming no room for COX-2 in the functional responses under such conditions. It is assumed that a decrease in acid secretion results in luminal alkalinization, which then contributes to maintenance of the microclimate for cellular restitution following barrier disruption (Svanes et al., 1981; Takeuchi et al., 1986). Certainly, other mediators such as nitric oxide (NO) and sensory neurons are also involved in the regulatory mechanism of gastric functional alterations following barrier disruption (Holzer et al., 1991; Takeuchi et al., 1993; 1994). However, since these factors interact with each other (Whittle et al., 1991), it is assumed that the lack of any one factor leads to failure of the full expression of these functional responses. We have previously shown that prednisolone, which is known to inhibit phospholipase A_2 activity, also inhibited functional changes in the stomach induced by hyperosmolar NaCl (Nobuhara et al., 1985). In any case, the present study together with previous findings suggest that local application of mild irritants such as TC first releases arachidonic acid from membrane phospholipids in association with the mucosal irritation and then increases PG production due to COX-1 activity, resulting in functional alterations to enhance the mucosal resistance to acid injury.

The COX-1 protein is ubiquitously expressed in various tissues in the gastrointestinal tract, including the stomach, while the expression of COX-2 protein is absent from most gastrointestinal tissues (Kargman et al., 1993). We also confirmed that COX-1 mRNA was expressed in the gastric mucosa, irrespective of whether or

not the stomach was exposed to TC. In contrast, the expression of COX-2 mRNA was not detected in the normal mucosa, and after exposure to TC remained undetectable for at least 90 min. As shown in the present study, the functional alterations were observed most markedly during or immediately after exposure to TC and subsided gradually after exposure, supporting the idea that COX-2 does not contribute to such responses occurring acutely in the stomach after barrier disruption. Certainly, the present study is acute in nature, and the results obtained will not be adequately applied in the stomach exposed to more prolonged irritation. Indeed, Barnett et al. (2000) reported the COX-2 expression in the stomach irritated with daily administration of 0.1% iodoacetamide for 5 days. We have previously reported that orally administered TC (20 mM plus 50 mM HCl) caused COX-2 induction in the stomach at 3 hr after administration and showed adaptive gastric protection mediated by endogenous PGs derived from both COX-1 and COX-2 (Yamamoto et al., 1999). It is also known that COX-2 plays a crucial role in the mechanism of ulcer healing (Mizuno et al., 1997, Araki et al., 2002). Furthermore, Wallace et al. (2000) reported that inhibition of both COX-1 and COX-2 is required for the gastric ulcerogenic properties of NSAIDs. The same is true for the intestinal ulcerogenic properties of NSAIDs (Tanaka et al., 2002). We also reported that selective COX-2 inhibitors alone provoke gastric lesions in rats with adjuvant-induced arthritis (Kato et al., 2002). Thus, it is assumed that both COX-1 and COX-2 play roles in maintaining the integrity of the gastrointestinal mucosa, yet the importance of these COX isozymes varies depending on the experimental conditions.

Given the above findings, the present study clearly showed that the COX-1 isozyme is involved in gastric functional responses, such as an increase of GMBF and a decrease in acid secretion, observed acutely after barrier disruption in the stomach. These functional alterations following barrier disruption are adaptive responses of

the stomach and play an important role in protecting the mucosa against acid injury by disposing of H^+ and maintaining a microclimate for cellular restitution. Since the stomach is continuously exposed to a variety of noxious stimuli such as acid, bile acids and food-related chemicals, it seems that endogenous PGs produced by COX-1 are crucial in maintaining adaptive functional responses of the stomach under adverse conditions.

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Figure Legends

Figure 1. A: Effects of various COX inhibitors on gastric mucosal PGE_2 content in rats. The animals were given indomethacin (5 mg/kg), SC-560 (5 mg/kg) or rofecoxib (5 mg/kg) p.o. and killed 3 hr later. **B:** Effects of these agents on PGE_2 content in the carrageenan-induced airpouch in rats. Indomethacin (5 mg/kg), SC-560 (5 mg/kg) or rofecoxib (5 mg/kg) was given p.o. 1 hr before the last injection of carrageenan in the pouch. Data are presented as the mean ± SE from 6 rats. ＊Significant difference from control, at $P<0.05$.

Figure 2. A: Effects of various COX inhibitors on changes in gastric PD after exposure of the stomach to TC in anesthetized rats. Animals were pretreated with omeprazole (60 mg/kg) to inhibit acid secretion. The stomach was exposed to 20 mM TC in 50 mM HCl for 30 min, and 50 mM HCl was applied to the stomach every 15 min before and after TC treatment. Indomethacin (5 mg/kg), SC-560 (5 mg/kg) or rofecoxib (5 mg/kg) was given i.d. 30 min before TC treatment. In **Figure B**, values indicate PD recovery (% recovery from the values observed immediately after TC treatment) in each group, and are presented as the mean±SE from 4-6 rats. ＊ Significant difference from control, at $P<0.05$.

Figure 3. Effects of various COX inhibitors on changes in GMBF after exposure of the stomach to TC in anesthetized rats. Animals were pretreated with omeprazole (60 mg/kg) to inhibit acid secretion. The stomach was exposed to 20 mM TC in 50 mM HCl for 30 min, and 50 mM HCl was applied to the stomach every 15 min before and

after TC treatment. Indomethacin (5 mg/kg), SC-560 (5 mg/kg) or rofecoxib (5 mg/kg) was given i.d. 30 min before TC treatment. Data are expressed as a percentage of basal values and represent the mean±SE of values determined every 15 min from 4-6 rats per group. Significant difference at $P<0.05$: # from basal values (time 0) in the corresponding group; ＊from control.

Figure 4. Effects of various COX inhibitors on changes in luminal acid loss in the stomach after exposure to TC in anesthetized rats. Animals were pretreated with omeprazole (60 mg/kg) to inhibit acid secretion. The stomach was exposed to 20 mM TC in 50 mM HCl for 30 min, and 50 mM HCl was applied to the stomach every 15 min before and after TC treatment. Indomethacin (5 mg/kg), SC-560 (5 mg/kg) or rofecoxib (5 mg/kg) was given i.d. 30 min before TC treatment. Data are presented as the mean±SE of values determined every 15 min from 4-6 rats per group. ＊Significant difference from basal values (time 0) in the corresponding group, at $P<0.05$. No significant difference was observed among four different groups.

Figure 5. Effects of various COX inhibitors on changes in gastric acid secretion after exposure of the stomach to TC in anesthetized rats. The stomach was exposed to 20 mM TC for 30 min, and was superfused with saline before and after the exposure. Indomethacin (5 mg/kg), SC-560 (5 mg/kg) or rofecoxib (5 mg/kg) was given i.d. 30 min before TC treatment. Data are presented as the mean±SE of values determined every 10 min from 4-6 rats per group. Significant difference at $P<0.05$: # from basal values (time 0) in the control group; ＊from control.

Figure 6. Development of gastric lesions in the anesthetized rat stomach after exposure to TC HCl, in the presence of various COX inhibitors. Animals were

pretreated with omeprazole (60 mg/kg) to inhibit acid secretion. The stomach was exposed to 50 mM HCl every 15 min before and after exposure to 20 mM TC in 50 mM HCl for 30 min and examined for damage 2 hr after TC treatment. Indomethacin (5 mg/kg), SC-560 (5 mg/kg) or rofecoxib (5 mg/kg) was given i.d. 30 min before TC treatment. Data are presented as the mean±SE from 4-6 rats. ＊ Significant difference from control, at $P<0.05$.

Figure 7. Effects of various COX inhibitors on changes in the mucosal PGE_2 content after exposure to TC in anesthetized rats. Animals were pretreated with omeprazole (60 mg/kg) to inhibit acid secretion. The stomach was exposed to 20 mM TC in 50 mM HCl for 30 min, and the mucosal PGE_2 content was measured 60 min after TC treatment. Indomethacin (5 mg/kg), SC-560 (5 mg/kg) or rofecoxib (5 mg/kg) was given i.d. 30 min before TC treatment. Data are presented as the mean±SE from 4~6 rats. Significant difference at $P<0.05$: ＊from normal; # from control.

Figure 8. Mucosal expression of COX-1 and COX-2 mRNAs in the stomach after exposure to TC in anesthetized rats. The stomach was exposed to 20 mM TC in 50 mM HCl for 30 min, and the expression of COX mRNAs was examined 30 and 90 min later. Figures show that the expression of COX-1 mRNA was consistently observed in the mucosa before and after exposure of the stomach to TC, while COX-2 mRNA was not detectable up to 90 min after TC treatment.

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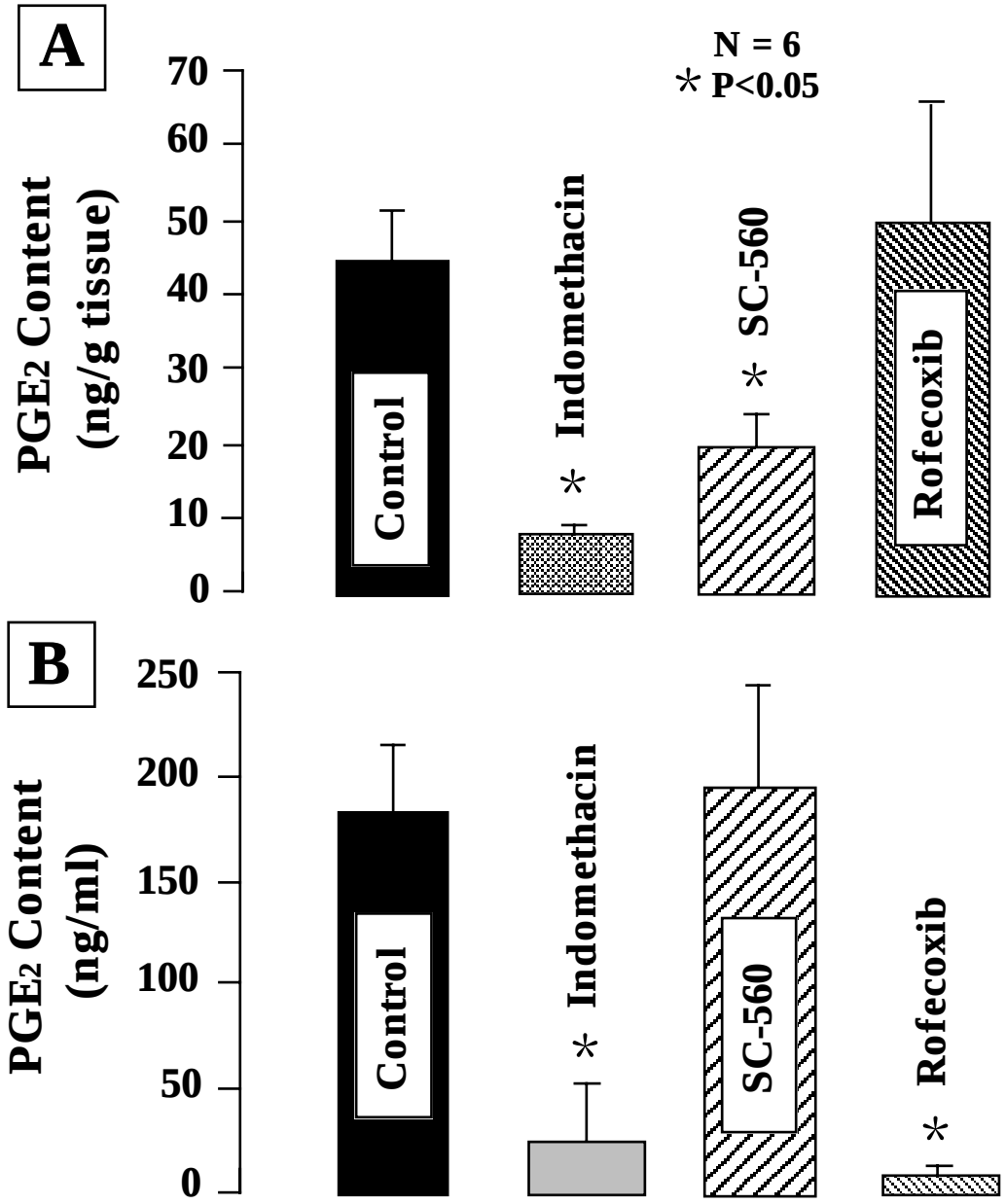

Figure 1

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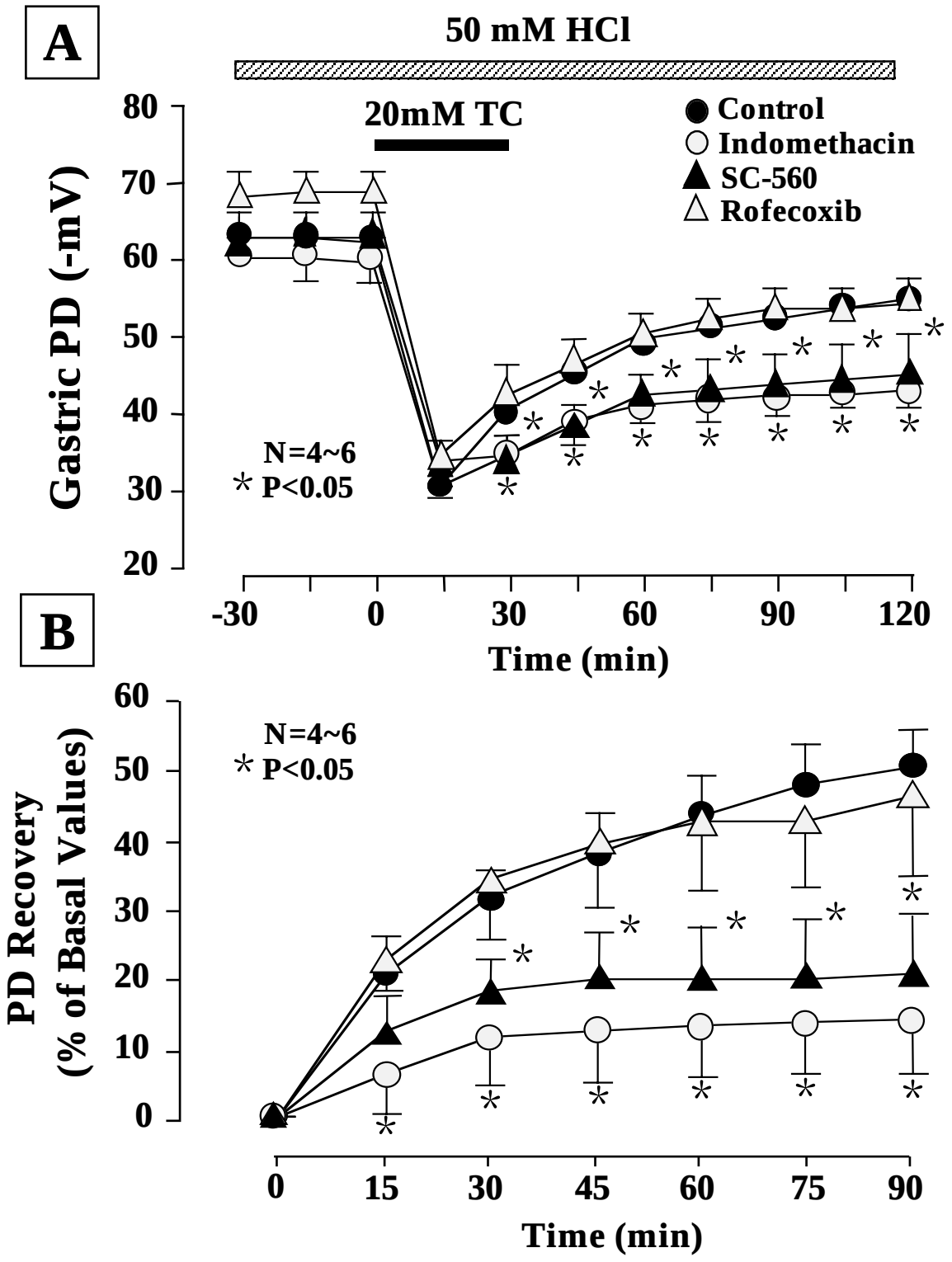

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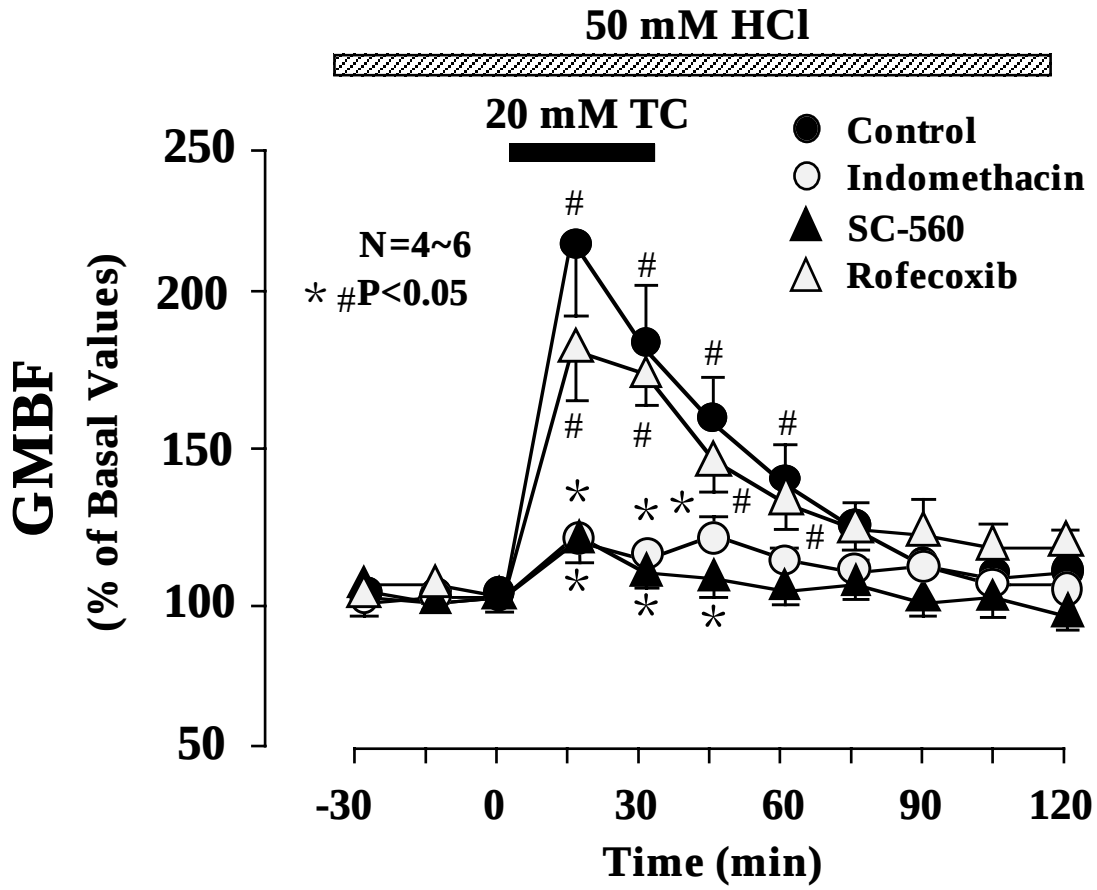

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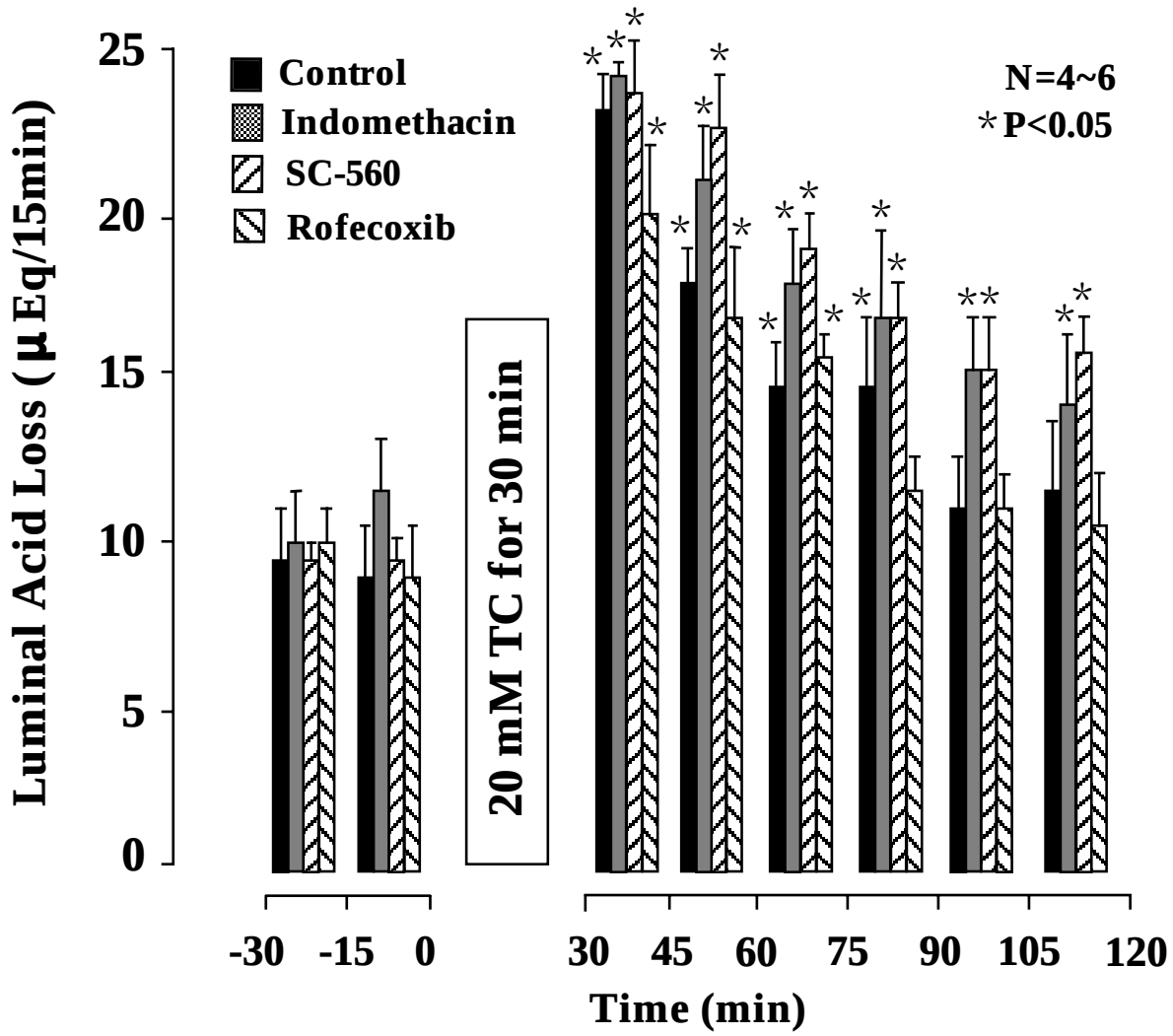

Figure 4

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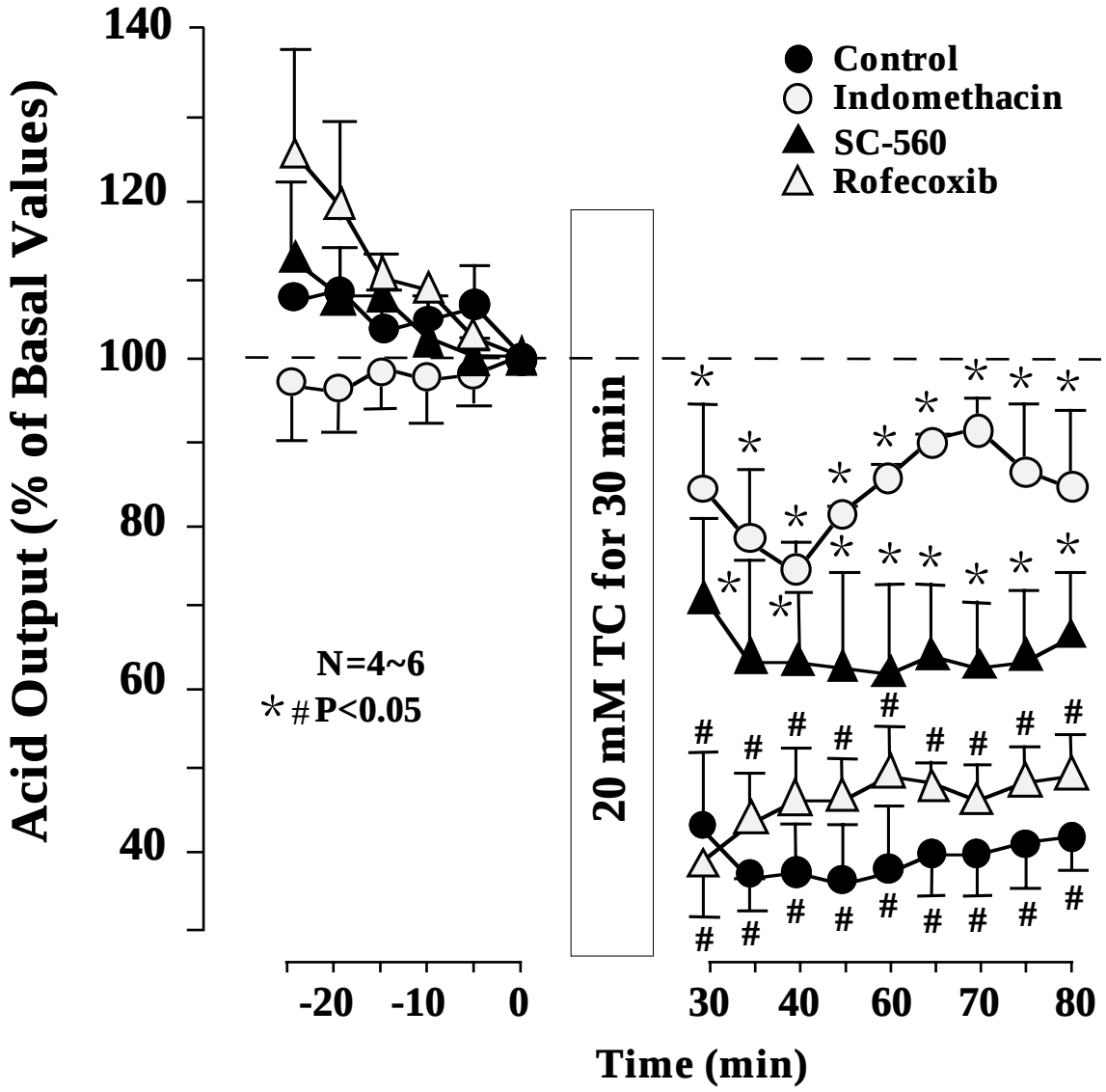

Figure 5

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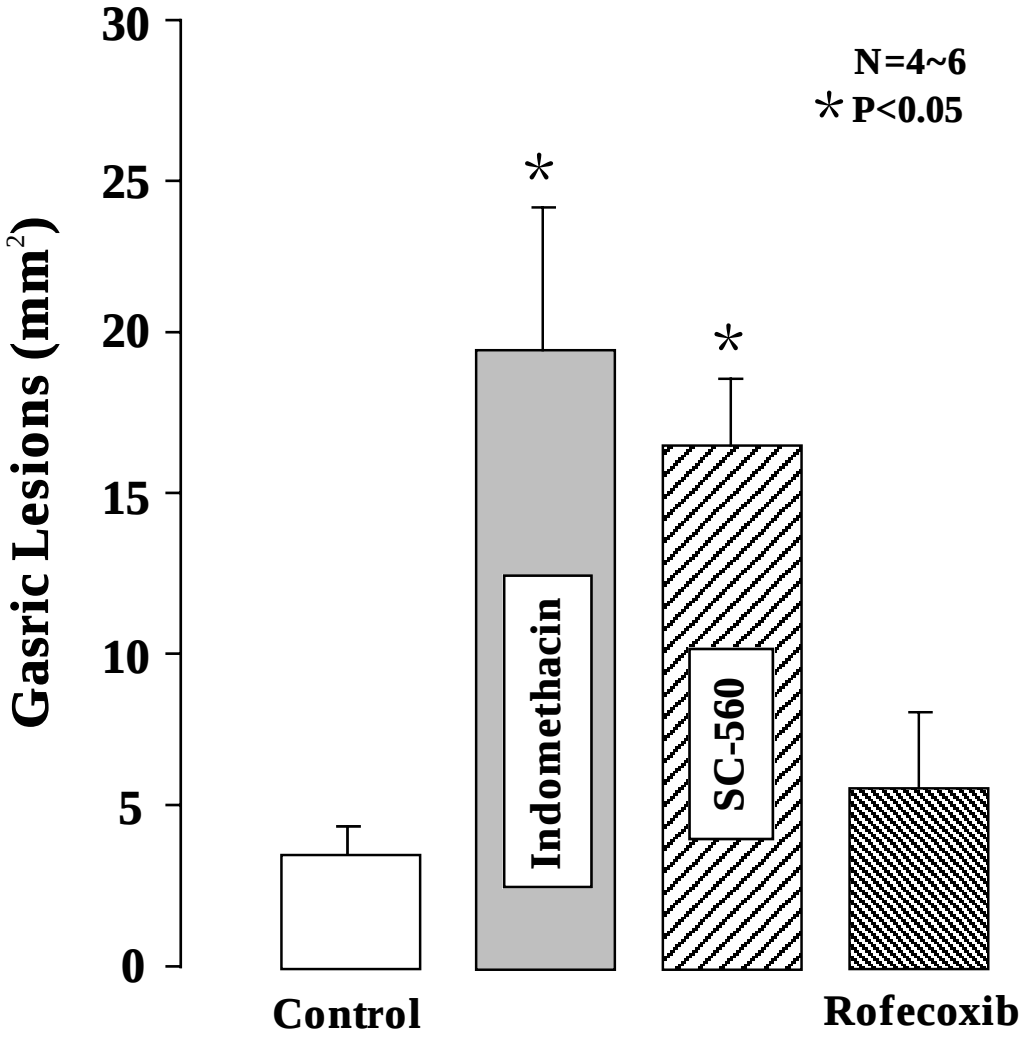

Figure 6

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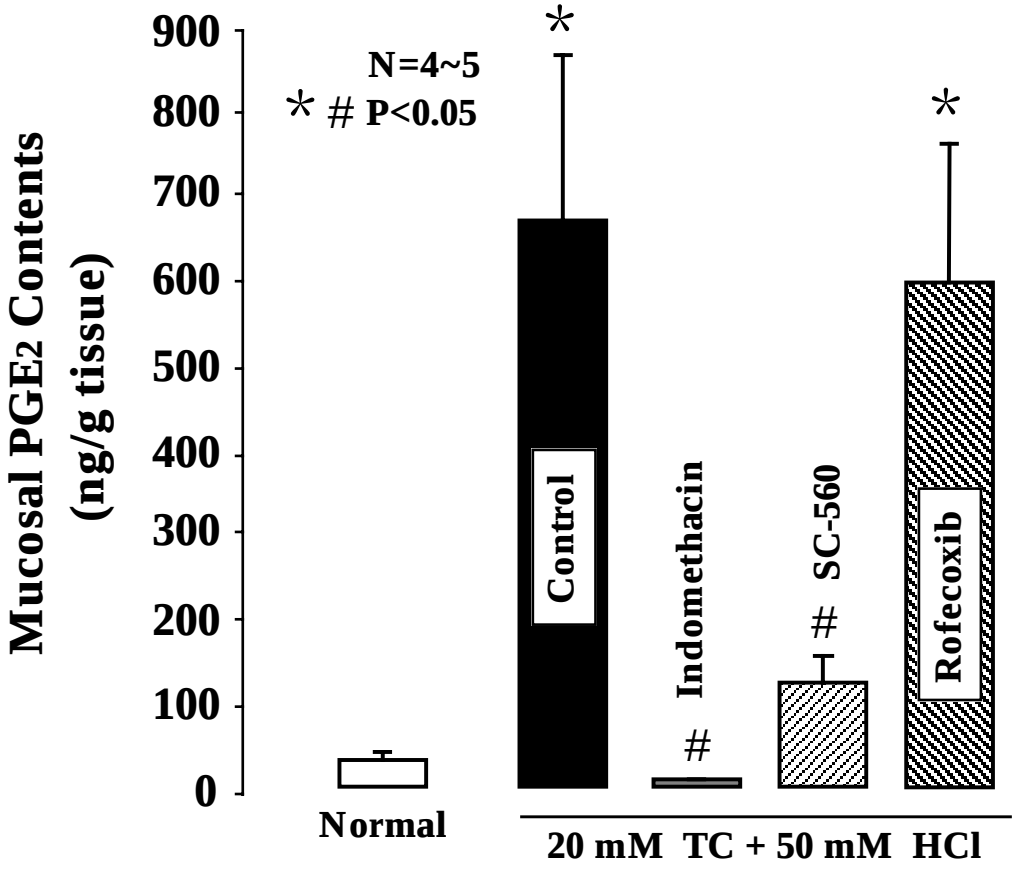

Figure 7

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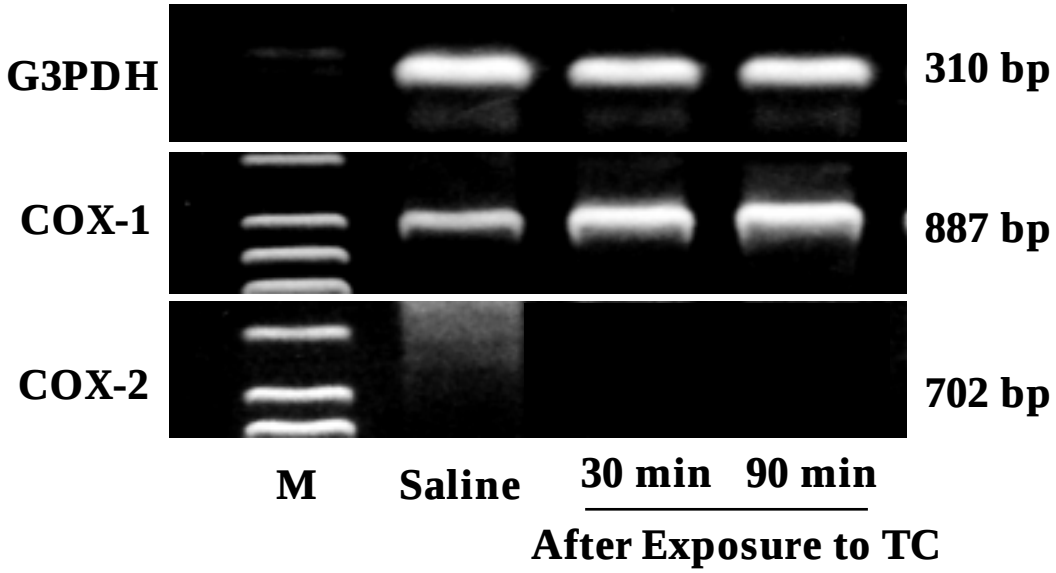

Figure 8