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Mechano-transcription of COX-2 is a common response to lumen dilation of the rat gastrointestinal tract

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Abstract

Background—In obstructive bowel disorders (OBDs) such as achalasia, pyloric stenosis, and bowel obstruction, the lumen of the affected segments is markedly dilated and the motility function is significantly impaired. We tested the hypothesis that mechanical stress in lumen dilation leads to induction of cyclo-oxygenase-2 (COX-2) in smooth muscle throughout the gastrointestinal (GI) tract, contributing to motility dysfunction.

Methods—Lumen dilation was induced in vivo with obstruction bands (12 × 3 mm) applied over the lower esophageal sphincter (LES), the pyloric sphincter, and the ileum in rats for 48 hr. Mechanical stretch in vivo was also emulated by balloon distension of the distal colon. Direct stretch of muscle strips from the esophagus, gastric fundus, and ileum was mimicked in an in vitro tissue culture system.

Key Results—Partial obstruction in the LES, pylorus, and ileum significantly increased expression of COX-2 mRNA and protein in the muscularis externae of the dilated segment oral to the occlusions, but not in the aboral segment. Direct stretch of the lumen in vivo or of muscle strips in vitro markedly induced COX-2 expression. The smooth muscle contractility was significantly suppressed in the balloon distended segments. However, treatment with COX-2 inhibitor NS-398 restored the contractility. Furthermore, in vivo administration of NS-398 in gastric outlet obstruction significantly improved gastric emptying.

Conclusions & Inferences—Mechanical dilation of the gut lumen by occlusion or direct distension induces gene expression of COX-2 throughout the GI tract. Mechanical stress-induced COX-2 contributes to motility dysfunction in conditions with lumen dilation.

Keywords

COX-2; Lumen dilation; Mechanical stress; Motility; Obstruction

Lumen dilation is a major pathological feature in many gastrointestinal disorders including achalasia, gastric outlet obstruction, bowel obstruction, pseudo-obstruction, and idiopathic mega-colon. Motility dysfunction is commonly encountered in these conditions. Achalasia is

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Conflicts of interest:

The authors disclose no conflicts.

characterized by impaired relaxation of the lower esophageal sphincter (LES) with dilated esophageal body, and suppressed peristaltic contractions [1-3]. In mechanical or functional obstructions in the small intestine and colon, motility dysfunctions are well documented [4-5]. These include delayed intestinal transit and decreased smooth muscle contractility [6-9]. Gastric dilation, as in the intestines, may be caused by mechanical obstruction or functional stasis. Mechanical gastric outlet obstruction happens most often in hypertrophic pyloric stenosis [10, 11]. Gastroparesis with delayed gastric emptying manifests as a functional gastric obstruction with increased gastric retention [12-15]. The gastric smooth muscle contractility is suppressed in gastroparesis [16, 17]. While motility dysfunctions in obstructive bowel disorders (OBDs) account for bloating, vomiting, dysphagia, abdominal distension, cramps, and constipation [1,2,4,5,10,13-15], the pathophysiological mechanisms underlying motility dysfunction in OBDs is not well understood. Investigations into the mechanisms for the impaired motility function in OBDs fragment each condition individually.

In the last decade or so, gastric banding has become one of the popular bariatric surgeries for obese patients. This surgery leads to marked short-term weight loss, largely due to early postprandial satiation and decreased food-intake due to the man-made obstruction near the esophageal-gastric junction [18]. However, recent data showed that gastric banding leads to marked esophageal dilation and motility dysfunction with weakened contractions in the esophageal body [19-21]. The mechanism for the motility dysfunction in gastric banding patients is not clear.

Lumen dilation has been considered a passive result of physical occlusion or functional stasis. However, we hypothesized that mechanical stress in lumen dilation leads to gene expression, which contributes to motility dysfunction. In a rat model of partial colon obstruction, we found that cyclooxygenase-2 (COX-2) was markedly up-regulated in the distended oral segment, but not in the segment aboral to obstruction band [9]. COX-2 and COX-2 –derived prostaglandins (PGs) have potent effects on motility function [9]. Based on the novel findings in the colon, we would like to determine whether mechanical stress-induced gene expression of COX-2 is a common mechanism throughout the GI tract and, if so, whether inhibition of COX-2 improves motility function in conditions with lumen dilation.

Methods and Materials

Rat models of obstruction in the esophagus, stomach, and ileum

Male Sprague-Dawley rats weighing 200-275 g and aged between 6 and 8 weeks (Harlan Sprague Dawley, Indianapolis, IN) were used for the study. The rats were housed in a controlled environment (22°C, 12-hr light-dark cycle) and allowed rodent pellet food and water ad libitum at all time unless stated otherwise. The Institutional Animal Care and Use Committee at University of Texas Medical Branch approved all procedures performed on the animals.

The rat models of obstruction were prepared by following procedures as previously described [7-9] with some modifications. Rats were anesthetized with 2% isoflurane

inhalation by an E-Z Anesthesia vaporizer (Palmer, PA). After midline laparotomy, the location where obstruction band was to be placed was carefully exposed. A small mesenteric window ($5 \times 5 \text{ mm}^2$) was made next to the exposed segment. To mimic lumen dilation encountered in the esophagus, stomach, and ileum, we applied medical grade silicon bands (3 mm wide) over the lower esophageal sphincter (LES), the pyloric sphincter, and the ileum (10 cm proximal to the ileum-colon junction) in separate rats (Fig. 1). The circumferences of the distal esophagus, the pylorus, and the ileum in rats were all less than 10 mm. The circumferences of the silicon bands were 12 mm in all these obstruction models, so that partial, but not complete, occlusion was achieved in each case. The sham control rats underwent the same surgical procedure except that the band was removed immediately after implantation. Unless stated otherwise, rats were euthanized 48 hours after operations.

NS-398 is a specific COX-2 inhibitor widely used in the *in vivo* animal studies [22, 23]. In experiments involving *in vivo* administration of NS-398, animal groups were randomly assigned to include sham surgery rats and obstruction rats each with vehicle (250 μL of 20% DMSO) or COX-2 inhibitor NS-398 (Cayman Chemical, Ann Arbor, MI) at 5 mg/kg in 250 μL of 20% DMSO, *i.p.* daily [22, 23]. NS-398 or vehicle was started one hour before the induction of obstruction. Once started, NS-398 or vehicle was given daily until the time of euthanasia.

In vivo stretch of distal colon with balloon distension—Mechanical stretch *in vivo* was emulated by balloon distension of the distal colon at 40 mmHg. Rats were anesthetized with 2% isoflurane inhalation by an E-Z anesthesia vaporizer, and a 6-cm long balloon attached to a catheter was inserted through the anus into the distal colon. The rats were then kept conscious in a container while the balloon was distended at 40 mmHg for 40 minutes. The pressure level of 40 mmHg was chosen because this level of balloon distension is generally considered not very noxious, but leads to lumen dilation in the colon [24-26]. Control rats were treated similarly in the lab except that no colonic balloon distension was applied.

Tissue collections—In the esophageal obstruction model, a 1 cm long segment of esophageal body was taken 1 cm oral to the LES. In the gastric outlet obstruction model, fundus tissue was taken for molecular and contractility studies. In some experiments, a 2 cm segment of small intestine (10 cm aboral to obstruction) was taken for control. In the model of ileal obstruction, a 2 cm long segment of ileum ~2 cm oral to obstruction was taken for the study. In some experiments, the ileal segment 2 cm aboral to obstruction was taken for control study. The tissue samples were collected in carbogenated Krebs buffer (in mmol/L: 118 NaCl, 4.7 KCl, 2.5 CaCl_2 , 1 NaH_2PO_4 , 1.2 MgCl_2 , 11 D-glucose, and 25 NaHCO_3). The segments were cleansed, opened along the mesenteric border, and pinned flat in Krebs buffer in a petri dish with Sylgard base. The mucosa/submucosa (M/S) and muscularis externa (ME) layers were separated by microdissection as described previously, and the ME was taken for the molecular measurements [9, 27-32].

In vitro stretch of smooth muscle strips—Smooth muscle strips ($3 \times 10 \text{ mm}$) were isolated from the esophagus, the gastric fundus, and the ileum, and cut along the long axis with the circular muscle orientation. To mimic mechanical stretch in obstruction *in vivo*, the

muscle strips were stretched to 130% of their original length and pinned on each end in DMEM+1% fetal bovine serum (FBS) (3 mL of media per strip) in a NuAire tissue culture system as described [9, 33]. The non-stretch control strips were kept at their original length with pins on each end.

Protein extraction and Western blots

The ME samples from the esophagus, gastric fundus, and ileum were homogenized on ice in lysis buffer supplemented with protease inhibitor cocktails (Sigma-Aldrich, St. Louis, MO) as described previously [9]. After spinning at 12,000 g at 4°C for 15 minutes, the supernatant proteins were collected and resolved by a standard immunoblotting method [9, 28, 29, 31-33]. Equal quantities (20 µg) of total protein were run on premade 4-12% Bis-Tris SDS-PAGE (Invitrogen, Carlsbad, CA). The primary antibody to COX-2 (1:1,000) was purchased from Cayman Chemical (Ann Arbor, MI). β -actin (1:5,000, Sigma, St. Louis, MO) was used as loading control. The protein detection was done using ODYSSEY Infrared Imaging System (LI-COR Biosciences, Lincoln, NE).

RNA preparation and real-time PCR

Total RNA was extracted from ME samples using the Qiagen RNeasy kit (Qiagen, Valencia, CA). One microgram of total RNA was reverse-transcribed using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) for real-time quantitative PCR with an Applied Biosystems 7000 real-time PCR system (Foster City, CA) [9, 29-32]. The assay ID for *Taqman* detection of rat COX-2 mRNA is Rn00568225-m1 (Applied Biosystems). For relative quantification of COX-2 gene transcription, real-time PCR was performed using 40 ng of cDNA for the target gene and for the endogenous control 18S rRNA (Part# 4352930E, Applied Biosystems).

Muscle bath experiments

The smooth muscle strips (3 × 10 mm) were mounted along the circular muscle orientation in individual muscle baths (Radnoti Glass, Monrovia, CA) filled with 10 ml carbogenated Krebs solution at 37°C. The contractile activity was recorded as previously described [9, 30-32] with Grass isometric force transducers and amplifiers connected to Biopac data-acquisition system (Biopac Systems, Goleta, CA). The muscle strips were first equilibrated in the muscle bath under 1 g tension for 60 min at 37°C. The smooth muscle contractility was tested by obtaining concentration-response curves to ACh (10^{-6} to 10^{-2} M). The contractile response, expressed as area under curves (AUC) was quantified as the increase in area under curves during 4 min after addition of ACh over the baseline area under curves during 4 min before the addition of ACh. The AUC of each individual strip ($\text{g}\cdot\text{sec}/\text{mm}^2$) was normalized by its cross section area (CSA). The CSA was determined using the following equation: $\text{CSA (mm}^2\text{)} = \text{tissue wet weight (mg)} / [\text{tissue length (mm)} \times 1.05 \text{ (muscle density in mg/mm}^3\text{)}]$.

Measurement of gastric emptying

Gastric emptying rate was determined as previously described [34] with minor modification. A 1.5 mL bolus of 1.5% methylcellulose (Fisher Scientific, Fair Lawn, NJ) containing 0.75

mg non-absorbable phenol red was gavage injected into the stomach. Gastric outlet obstruction led to marked retention of gastric contents. To keep the difference of gastric contents between sham and obstruction rats to a minimum, rats were allowed free access to food and water before injection of phenol red. However, all rats were deprived of food access after the injection of phenol red. Sixty minutes after the gavage injection, rats were euthanized by CO₂ inhalation. The whole stomach was removed immediately, and placed in 25 mL of 0.1N NaOH and homogenized. The homogenate was kept at room temperature for 1 hr. One milliliter of the supernatant was added to 0.1 mL of 20% trichloroacetic acid (TCA) solution to precipitate the protein. After centrifugation at 10,000g for 15 min, 1 mL of 0.5N NaOH was added to the supernatant. The amount of phenol red was determined by measuring the absorption at 550 nm using an Eppendorf Biophotometer Plus (Eppendorf North America, Inc. Westbury, N.Y.).

Statistical analysis

All data points are expressed as means $\pm$ SEM. Statistical analysis was performed by analysis of variance with non-repeated measures (by Student-Newman-Keuls test) for comparisons of multiple groups, and Student's t-test for comparisons of two groups. A *p* value of 0.05 was considered statistically significant.

Results

1. In vivo model of lumen dilation in the esophagus by obstruction band

After an obstruction band was placed at the LES, the esophageal body became dilated over time. The circumference of the esophageal body (1 cm proximal to the LES) was 11.2 ± 1.3 mm in the obstructed rats compared to 7.6 ± 0.2 mm in the sham controls 48 h after operations ($p < 0.05$, $n = 5$). Partial obstruction in the LES led to significant up-regulation of COX-2 protein and mRNA expression in the esophageal body. The COX-2 mRNA and protein levels in the esophageal body increased by $1.8(\pm 0.2)$ -fold and $7.8(\pm 2.3)$ -fold, respectively, compared to the sham ($n=4$ or 5 , both $p < 0.05$) 48 h after the introduction of the obstruction band (Fig. 2A, 2B).

2. In vivo model of lumen dilation in the ileum by obstruction band

When an obstruction band was placed around the ileum 10 cm proximal to the ileum-colon junction, the ileal segment oral to the obstruction band became dilated, and the COX-2 expression in the ileal ME was markedly increased (Fig. 2C, 2D). After 48 h of obstruction, the COX-2 mRNA and protein levels increased by $25.6(\pm 2.1)$ -fold and $11.1(\pm 0.8)$ -fold, respectively ($n = 4$, both $p < 0.01$). On the contrary, the COX-2 level in the aboral segment did not change (Fig. 2C, 2D).

3. In vivo model of lumen dilation in the stomach by gastric outlet obstruction

Partial obstruction of the pyloric sphincter led to marked dilation of the stomach. The overall stomach weight (the stomach and intraluminal contents) at euthanasia was 7.2 ± 0.3 g in the sham operated rats, and 12.9 ± 0.8 g in the outlet obstructed rats ($p < 0.01$, $n = 4$). After 48 h of obstruction, the COX-2 mRNA and protein levels increased by $12.5(\pm 3.8)$ -fold and $10.0(\pm 1.5)$ -fold, respectively in the fundus tissue ($n = 4$, Fig. 3). However, the COX-2

expression in the un-dilated jejunum (caudal to the obstruction band) did not change (Fig. 3).

4. Administration of COX-2 inhibitor in gastric outlet obstruction

To determine if mechanical stress-induced COX-2 plays a role in motility dysfunction in gastric distension, COX-2 inhibitor NS-398 was administered in rats with sham operation and with obstruction. The gastric emptying and gastric fundus contractility were measured 2 days after gastric obstruction (Fig. 4A). The gastric emptying was $70.5(\pm 2.5)\%$ in the control rats 60 minutes after the injection of phenol red. Outlet obstruction markedly decreased gastric emptying to $13.0(\pm 10.6)\%$ ($p < 0.05$, $n = 4$). However, NS-398 treatment significantly improved gastric emptying to $41.3(\pm 6.7)\%$ in the obstruction rats. NS-398 treatment in control rats had a trend to decrease gastric emptying [$46.1(\pm 8.6)\%$, $p = 0.063$, $n=4$], although the difference is not statistically significant compared to sham with vehicle (Fig. 4A).

Muscle bath study showed that gastric dilation in outlet obstruction suppressed fundus circular muscle contractility in response to acetylcholine (ACh, 10^{-6} to 10^{-2} M) (Fig. 4B, 4C). However, NS-398 treatment significantly restored smooth muscle contractility in obstruction. NS-398 had no significant effect on muscle contractility in sham control rats ($n=4$).

5. In vivo model of lumen distension by a balloon in the distal colon

In the balloon distension model, the distal colon was distended with a balloon at 40 mmHg for 40 minutes, and the rats were euthanized 24 hr later. The COX-2 mRNA and protein levels were significantly increased by $3.6(\pm 0.3)$ -fold and $27.3(\pm 8.4)$ -fold, respectively, in the ME of the distended colons compared to controls (both $p < 0.05$, $n = 4$) (Fig. 5A and 5B).

To determine if mechanical stress-induced COX-2 plays a role in colonic SMC contractility, circular muscle strips were isolated from the distal colons of control rats and rats treated with balloon distension 24 hr earlier, and incubated in vehicle (DMSO) or NS-398 ($1 \mu\text{M}$) in DMEM+1% FBS for 24 h. $1 \mu\text{M}$ of NS-398 effectively blocked COX-2 activity in the colon ME [9]. Muscle bath experiments showed that the colonic circular muscle contractility was significantly suppressed in the distended rats compared to controls. However, the smooth muscle contractility was significantly improved by incubation with NS-398 (Fig. 5C).

6. In vitro stretch of smooth muscle strips

Direct stretch of the muscle strips from the esophagus, gastric fundus, ileum, and colon significantly induced expression of COX-2 protein and mRNA in all the tissues (Fig. 6). The COX-2 mRNA expression increased 3.1 ± 0.8 , 4.2 ± 1.6 , 2.1 ± 0.4 , and 10.0 ± 2.4 -fold in the stretched circular muscle strips isolated from the esophagus, gastric fundus, ileum, and colon, respectively (All $p < 0.05$ vs non-stretch control, $n = 5$ or 6).

Discussion

Etiologies for OBDs such as achalasia, gastric outlet obstruction (pyloric stenosis), gastroparesis, bowel obstruction, and pseudo-obstruction may be very different. However lumen dilation is a common pathological feature, and motility function is impaired in all of these conditions. The impaired motility function is associated with symptoms such as bloating, vomiting, abdominal distension, pain, and constipation. As for the relationship between lumen dilation and motility dysfunction in OBDs, lumen dilation has been considered as a passive result of mechanical occlusion or functional stasis. Although this may be true at the beginning of a pathological condition, we now found that lumen dilation significantly alters gene expression in gut smooth muscle. COX-2, a mechanically sensitive molecule, is up-regulated in the models of lumen distension in the esophagus, stomach, small intestine, as in the colon [9]. Furthermore, our study showed that mechanical stress-induced COX-2 plays a critical role in motility dysfunction in lumen dilations in rats. Treatment of the smooth muscle tissue from the distended lumen with COX-2 inhibitors significantly restored muscle contractility. Administration of COX-2 inhibitors *in vivo* in gastric outlet obstruction prevented impairments of smooth muscle contractility, and improved gastric emptying. Previously, we have demonstrated that COX-2 inhibitor has prophylactic and therapeutic benefits for motility dysfunction in the obstructed colons [9, 32].

The causes of mechanical lumen dilations may be due to occlusions extrinsic to the gut, e.g. adhesions or strangulation, or may originate intrinsic to the lumen, e.g. tumors and diverticulitis. In laboratory animals, lumen dilation has been reproduced by external application of a silicon band [7-9], or by inflation of an intraluminal balloon [6, 35]. In the present study, we found that no matter the method, lumen dilation leads to gene expression of COX-2 in the alimentary tract.

To make models of esophageal, gastric, and ileal obstruction in rats, we exercised great care. A small mesenteric window was made next to the segment for the placement of the obstruction band. This avoided unnecessary contact with the segment to be studied. In addition, strict sham controls were kept for each model. The sham control rats underwent the same surgical procedure except that the band was removed immediately after implantation. We found that pyloric banding induced expression of COX-2 in the gastric tissue but not in the jejunum. LES banding in the abdominal cavity led to COX-2 expression of the esophageal body, which is located in the thoracic cavity. Furthermore, direct stretch of the lumen with a balloon *in vivo*, or smooth muscle strips *in vitro* led to marked expression of COX-2 in the smooth muscle. In the primary culture of colonic SMCs, mechanical stretch induces marked expression of COX-2 mRNA and proteins [9] and release of PGE₂ (Lin et al, unpublished observation). These data demonstrate that expression of COX-2 in our models of lumen distension is induced by mechanical stress in the gut, and that the COX-2 gene is a mechanically responsive gene in the GI tract. Recent study found that other mechanically responsive genes, such as those encoding E-prostaglandin receptor EP2, may also be induced in lumen dilation [32]. Our cDNA microarray study found that mechanical force has specific effects on gene expression in SMCs and neuroplasticity in the gut. Unlike COX-2, the expression of COX-1 is not altered in bowel obstruction [9]. An understanding

of other mechanically responsive genes in the GI tract may further delineate pathophysiologies encountered in OBDs. However, the present study serves to propose a common mechanism “mechanotranscription” in different parts of the GI tract, taking COX-2 only as an example.

How COX and COX-derived PGs affect smooth muscle contractions is an active field of research. It has been shown that PGE₂ at physiological level helps to maintain gut smooth muscle contractility through EP1 and EP3 receptors in normal state [32]. However, abnormally increased PGE₂ in pathologies such as obstruction or inflammation suppresses smooth muscle contraction via EP2 and EP4 receptors [9, 23, 32]. This discrepancy may help to explain why NS-398 had a trend to slow gastric emptying in control rats, but significantly improved motility function in obstructed rats in our study.

Gastric dilation is a direct consequence of gastric outlet obstruction as in hypertrophic pyloric stenosis [10, 11], and a notable feature in gastroparesis [12, 15]. An 8-year barium radiographic study found that 60% of gastroparesis patients had gastric dilation [15]. Delayed gastric emptying and impaired smooth muscle contractility in gastroparesis are responsible for symptoms such as bloating, vomiting, and abdominal distension. Several drugs including metoclopramide, domperidone, tegaserod and other 5-HT₃ agonists, erythromycin and its derivatives are among the therapeutics for gastroparesis [36, 37]. However, the efficacy of these prokinetics is largely unsatisfactory [36, 37]. It is noteworthy that all these drugs act to stimulate smooth muscle contractions through increased release of excitatory neurotransmitter acetylcholine or by directly acting on receptors on smooth muscle cells [38-41]. Our data show that the expression of a motility-suppressing molecule, COX-2, is markedly up-regulated in gastric dilation. Thus, inhibition of mechanical stress-induced COX-2 and other mechanically responsive genes may represent a novel therapeutic potential in patients with gastric dilation. Inhibition of mechanotranscription-initiated suppression of SMC contractility in such conditions would be complementary to the action of prokinetics to stimulate SMC contractions.

Achalasia is associated with an increased incidence of esophageal carcinoma [42-44]. The reason is not clear. However, it is shown that COX-2 plays an important role in the development of esophageal carcinoma [45, 46] and COX-2 inhibitors are effective in preventing esophageal cancer [45-47]. Our finding that mechanical stress in esophageal dilation induces COX-2 expression may offer an explanation why achalasia patients have a much higher risk for esophageal cancer.

Over the last decade or so, gastric banding has become an appealing surgical option to manage obesity [18]. However, the man-made obstruction near the esophageal-gastric junction may lead to esophageal dilation and motility dysfunction [19-21]. The long-term impacts of gastric banding are not well known because of a relatively short history of this procedure. However, there have been emerging reports of precancerous [48] and cancerous esophageal conditions [49-51] in the gastric banding patients, who did not experience such abnormalities before surgery. As gastric banding may lead to esophageal dilation [19, 20] which induces expression of COX-2, the clinical relevance of mechanotranscription in gastric banding deserves further studies.

Taken together, our study showed that lumen dilation markedly induces gene expression of COX-2 in the smooth muscle of the esophagus, stomach, and intestines in the obstruction models. Direct stretch of the lumen in vivo with a balloon or of the smooth muscle strips in vitro also significantly induces COX-2 expression. Furthermore, treatment with COX-2 inhibitor NS-398 significantly restores smooth muscle contractility and improves motility function in lumen dilation. Our study therefore demonstrates that mechanical stress-induced COX-2 is a common phenomenon in conditions with lumen dilation throughout the GI tract. Mechanical stress-induced COX-2 contributes to motility dysfunction in obstructive conditions in the gut.

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Abbreviations

COX-2	cyclooxygenase-2
GI	gastrointestinal
LES	lower esophageal sphincter
ME	muscularis externa
OBD	obstructive bowel disorder
PGE₂	prostaglandin E ₂
SMC	smooth muscle cell

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A. Models of obstructions in the upper gut**B. Balloon distension** **Fig. 1. Diagram of lumen dilations in obstruction and balloon distension**

(A) Medical grade silicon bands (12 mm long, 3 mm wide) were applied over the lower esophageal sphincter (LES), the pyloric sphincter, and the ileum of 6~8 weeks old Sprague-Dawley rats to mimic lumen dilation encountered in esophageal obstruction (EO), gastric outlet obstruction (GO), and ileal obstruction (IO). The circumferences of the bands were 12 mm, so that partial, but not complete, occlusion was achieved in each case. Sham operated rats served as control (shown on the left for each model). (B) Balloon distension (BD) was achieved with a balloon placed in the distal colon at 40 mmHg for 40 min.

A. Expression of COX-2 protein in EO **C. Expression of COX-2 protein in IO** **B. Expression of COX-2 mRNA in EO** **D. Expression of COX-2 mRNA in IO**

Fig. 2. Expression of COX-2 protein and mRNA in esophageal obstruction (A, B) and ileum obstruction (C, D)

A medical grade silicon band was applied at the lower esophageal sphincter (LES) for esophageal obstruction (EO) or at the ileum (10 cm proximal to ileum-colon junction) for ileal obstruction (IO). Rats were euthanized 48 h after operations for the detections of COX-2 protein and mRNA. Sham operated rats served as control. Numbers in the parentheses are the n values of animals used in the group, * $p < 0.05$ vs. sham.

A. COX-2 protein expression in GO

B. COX-2 mRNA expression in GO

Fig. 3. Expression of COX-2 protein (A) and mRNA (B) in gastric outlet obstruction (GO)
 A medical grade silicon band (3 mm wide) was applied at the pyloric sphincter in rats to mimic gastric outlet obstruction (GO). Sham operated rats served as control. Rats were euthanized 48 h after operations. Note that when gastric outlet is obstructed, the expression of COX-2 mRNA and protein is increased in the gastric fundus (Fund.). However, COX-2 expression is not increased in the jejunum (Jej.), which is aboral to the obstruction band. Numbers in the parentheses are the n values of animals used in the group. * $p < 0.05$ vs. sham control in the same tissue group.

A. Gastric emptying

B. Smooth muscle contractility

C. Trace of smooth muscle contractile response

Fig. 4. Effect of in vivo administration of COX-2 inhibitor NS-398 on gastric emptying (A) and fundus circular muscle contractility (B, C) in gastric outlet obstruction (GO)

Rats with sham operation and GO were treated with vehicle (DMSO) or NS-398 (5 mg/kg, i.p., daily). Rats were euthanized 48 hours after operations. Gastric emptying was determined as described in the methods and data is summarized in (A). In separate experiments, circular muscle strips were isolated from the gastric fundus for the determination of muscle contractile response to acetylcholine (ACh, 10^{-6} to 10^{-2} M) in a muscle bath. Quantitative data are shown in (B) as % change of area under curves (AUCs) normalized by cross section area relative to the maximal response in sham/vehicle rats. Representative traces of smooth muscle contractile response to ACh in each of the 4 groups were shown in (C). Numbers in the parentheses in (A) and (B) are the n values of animals used in the group. Numbers in the parentheses in (C) are the cross section area (in mm^2) for each muscle strip. * $p < 0.05$ vs. sham control, # $p < 0.05$ vs. obstruction treated with vehicle.

A. COX-2 protein **B. COX-2 mRNA** **C. Circular muscle contractility in muscle bath** **Fig. 5. Balloon distension in the distal colon**

Balloon distension (BD) was achieved with a balloon placed in the distal colon at 40 mmHg for 40 minutes. Age-matched control rats underwent all the similar laboratory procedures as in the BD rats, except that no balloon distension was applied. Rats were euthanized 24 hours later for tissue-taking for the detections of COX-2 protein (A) and COX-2 mRNA (B). To determine if mechanical stress-induced COX-2 plays a role in colonic SMC contractility, distal colon muscle strips from the sham and distended rats were incubated in DMEM with vehicle (DMSO) or NS-398 (1 μ M) for 24 h before the smooth muscle contractility was determined in muscle bath (C). Quantitative data are shown in top panel of (C) as % change of area under curves (AUCs) normalized by cross section area relative to the maximal response in control/vehicle rats. Representative traces of smooth muscle contractile response to ACh in each of the 4 groups were shown in the bottom panel of (C). Numbers in the parentheses in (A), (B), and top panel of (C) are the n values of animals used in the group. Numbers in the parentheses in the bottom panel of (C) are the cross section area (in mm²) for each muscle strip. * $p < 0.05$ vs. control/vehicle. # $p < 0.05$ vs. BD treated with vehicle.

A. Esophagus **B. Gastric fundus** **C. Ileum** **D. Colon**

Fig. 6. Direct stretch of circular muscle strips in vitro from the esophagus (A), gastric fundus (B), ileum (C), and distal colon (D) induces expression of COX-2 protein and mRNA

Muscle strips were cultured in DMEM + 1% FBS for 24 h in silicon elastomer-bottomed plates. Strips (3 mm-width) were stretched to 130% of the original length with a pin placed at each of the two ends. The non-stretch control strips were treated similarly except that the stretch was not applied. Numbers in the parentheses are the n values of tissue strips used in the group. * $p < 0.05$ vs. non-stretch control. Non-S, non-stretch control; Str, stretched.