Prostaglandins mediate tonic contraction of the guinea pig and human gallbladder

Ping Cong, Zuo-Liang Xiao, Piero Biancani, and Jose Behar

Prostaglandins mediate tonic contraction of the guinea pig and human gallbladder. Am J Physiol Gastrointest Liver Physiol 292: G409–G418, 2007. First published June 8, 2006; doi:10.1152/ajpgi.00091.2006.—The gallbladder (GB) maintains tonic contraction modulated by neurohormonal inputs but generated by myogenic mechanisms. The aim of these studies was to examine the role of prostaglandins in the genesis of GB myogenic tension. Muscle strips and cells were treated with prostaglandin antagonists, agonists, cyclooxygenase (COX) inhibitors, and small interference RNA (siRNA). The results show that PGE2, thromboxane A2 (TxA2), and PGF2α cause a dose-dependent contraction of muscle strips and cells. However, only TxA2 and PGE2 (E prostanoid 1 receptor type) antagonists induced a dose-dependent decrease in tonic tension. A COX-1 inhibitor decreased partially the tonic contraction and TxB2 (TxA2 stable metabolite) levels; a COX-2 inhibitor lowered the tonic contraction partially and reduced PGE2 levels. Both inhibitors and the nonselective COX inhibitor indomethacin abolished the tonic contraction. Transfection of human GB muscle strips with COX-1 siRNA partially lowered the tonic contraction and reduced COX-1 protein expression and TxB2 levels; COX-2 siRNA also partially reduced the tonic contraction, the protein expression of COX-2, and PGE2. Stretching muscle strips by 1, 2, 3, and 4 g increased the active tension, TxB2, and PGE2 levels; a COX-1 inhibitor prevented the increase in tension and TxB2; and a COX-2 inhibitor inhibited the expected rise in tonic contraction and PGE2. Indomethacin blocked the rise in tension and TxB2 and PGE2 levels. We conclude that PGE2 generated by COX-2 and TxA2 generated by COX-1 contributes to the maintenance of GB tonic contraction and that variations in tonic contraction are associated with concomitant changes in PGE2 and TxA2 levels.

gallbladder; muscle tension; prostaglandin E2; thromboxane A2; COX-1; COX-2; siRNA

The gallbladder (GB) maintains tonic contraction in vivo and in muscle strips in vitro (4, 14). It is regulated by excitatory cholinergic neurons and by inhibitory adrenergic neurons by stimulating β-adrenergic receptors (3, 12). However, the tonic contraction is not affected by functional denervation with TTX, suggesting that a major component of the tonic contraction is myogenic. The mechanisms involved in the generation of this myogenic contraction, however, have not been elucidated.

The functions of prostaglandins, particularly prostaglandin E2 (PGE2), have been traditionally studied for their role in cytoprotection and inflammatory processes (17, 44, 53, 50). PGE2 levels are increased in acute and chronic cholecystitis (30, 48). PGE2 also increases in response to oxidative stress to stimulate catalase, a scavenger of reactive oxygen species, to attenuate their deleterious effects on proteins and lipids (50).

Prostaglandins may also contribute to the genesis of tonic contraction in a variety of smooth muscles. Three major prostaglandins have been investigated, with prostaglandin F2α (PGF2α) and thromboxane A2 (TxA2) being the major candidates. PGE2, TxA2, and PGF2α contract vascular and bronchial muscle cells (7, 5, 16, 20, 22, 25, 40, 46). PGF2α and TxA2 also contribute to the tonic contraction of the cat lower esophageal sphincter because it is inhibited by indomethacin by PGF2α and TxA2 receptor antagonists (10). It has also been shown that PGE2 contributes to the tonic contraction of certain types of muscle cells. However, it relaxes gastrointestinal circular muscle cells (8), but it contracts muscle cells from the longitudinal muscle layer of the colon (47).

Prostaglandins are uniquely suited to perform these muscle functions. Muscle cells generate prostaglandins and export them to the extracellular space where they act as autacoids or local hormones. These endogenous prostaglandins may regulate smooth muscle tonic contraction by stimulating specific G protein-coupled receptors (1), are capable of stimulating Rho kinase (38), and therefore may be able to generate a sustained or tonic contraction (29).

Endogenous TxA2 activates thromboxane prostanoid (TP) receptors that play a physiological role in the regulation of spontaneous contractile activity in the porcine uterus (9) and human penile arterial and trabecular smooth muscle (1). The actions of PGE2, however, are more complex because it can activate four sets of E prostanoid (EP) receptors (6, 44). EP1 and 3 mediate contraction (25, 40, 45, 54), whereas EP2 and 4 mediate relaxation (1, 18). Thus the muscle response to PGE2 depends on the population of receptors present in each muscle cell type. Moreover, more than one prostaglandin may be involved in the maintenance of muscle tension. The contractions of human vascular smooth muscle induced by prostanoids involved both TP and EP1 receptors (23, 45).

The present studies were aimed at examining whether prostaglandins participate in the genesis of GB tonic contraction.

MATERIALS AND METHODS

Patients. Human GB with pigment stone were obtained by elective laparoscopic cholecystectomy performed for gallstone disease. None of the patients had a clinical or histological evidence of acute cholecystitis. Only GB with pigment stones were included in these studies and were determined according to their gross appearance and chemical analysis (4, 11, 13). These GB specimens were selected because they had no evidence of histological inflammation or biochemical parameters of oxidative stress (4, 53).

Address for reprint requests and other correspondence: J. Behar, Div. of Gastroenterology, APC 406, Rhode Island Hospital/Brown Univ. Medical School, 593 Eddy St., Providence, RI 02903 (e-mail: Jose_Behar@brown.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpgi.org 0193-1857/07 $8.00 Copyright © 2007 the American Physiological Society G409
Animals. Male guinea pigs (weight 450–500 g) were purchased from Charles River Laboratory (Wilmington, MA). The Animal Welfare Committee of Rhode Island Hospital has approved their use. Animals were housed in thermoregulated rooms with free access to food and water. After an overnight fast, the guinea pigs were sedated with an intramuscular injection of ketamine hydrochloride (30 mg/kg) and euthanized by pentobarbital (30 mg/kg ip).

Both human and guinea pig GB were promptly removed, rinsed with ice-cold, oxygenated Krebs solution (11, 13, 53, 55) and placed in a dissecting pan containing the same solution continuously aerated with 95% O2–5% CO2. The GB was kept in ice-cold oxygenated Krebs solution (116.6 mM NaCl, 3.4 mM KCl, 21.9 mM NaHCO3, 1.2 mM NaH2PO4, 2.5 mM CaCl2, 1.2 mM MgCl2, 5.4 mM glucose). The mucosa and serosa were carefully peeled off under a dissecting microscope. Gently removing the remaining connective tissue further cleaned the GB muscle layer.

Isolation and permeabilization of GB muscle cells. Muscle cells were isolated, and in some experiments they were permeabilized by methods described previously (13, 50–52, 55). GB muscle layer was cut into 2-mm-wide strips and digested in HEPES buffer containing 0.5 mg/ml type F collagenase and 2 mg/ml papain (activity of 13.9 U/mg protein) for 20 min at 35°C in a shaking water bath. The HEPES buffer consisted of 112.5 mM NaCl, 5.5 mM KCl, 2.0 mM CaCl2, 2.0 mM KH2PO4, 24 mM HEPES, 1.9 mM CaCl2, 0.6 mM MgCl2, and 10.8 mM glucose. The buffer was gently gassed with 100% O2 during the tissue digestion. At the end of the digestive process, the tissue was filtered through Nitex mesh 200 (Tetko, Elmsford, NY) and rinsed with 20 ml of HEPES. The tissue remaining on the filter was collected and incubated in HEPES buffer at 35°C for 15 min to allow free dispersion of cells. For preparation of permeable muscle cells, the partly digested tissues were washed with cytosolic buffer and exposed briefly to saponin (75 µg/ml) during centrifugation at 200 g for 3 min. Cells were washed and resuspended in modified cytosolic buffer for further use.

Studies on muscle cell contraction. Muscle contraction was measured as previously described (13, 50, 51, 55, 56). Intact and permeable cells were treated with increasing concentrations of agonists for 30 s and then fixed in acrolein at 1% final concentration. The cell length was measured with a phase-contrast microscope (Carl Zeiss, Jena, Germany) and a closed-circuit television camera (Panasonic, Secaucus, NJ) connected to a Macintosh computer with NIH Image software. The average length of 30 cells, measured in the absence of agonists, was taken as the “control” length and compared with length measured after addition of agonists. Shortening was defined as the percentage decrease in the average length after treatment with agonists compared with the control length.

Preparation of plasma membranes. Plasma membranes were prepared and purified by sucrose gradient centrifugation, as described previously (39, 49, 50, 51). Muscle cells were homogenized by using a tissue tearer (Biospec Products, Racine, WI) in 10 volumes by weight of a sucrose-HEPES buffer. The homogenates were centrifuged at 600 × g for 20 min at 35°C in a shaking water bath. The supernatant was determined. Then the supernatants were subjected to SDS-PAGE, and the separated proteins were electrophoretically transferred to a nitrocellulose membrane at 30 V overnight. The nitrocellulose membranes were blocked in 5% nonfat dry milk and then incubated with a nitrocellulose antibody at 30°C for 2 h, the wells were washed three times with phosphate buffer and then placed in serum- and antibiotic-free RPMI 1640 medium containing scramble, COX-1 or COX-2 siRNA (100 pmol) and lipofectamine 2000 (Invitrogen, Carlsbad, CA) as instructed by the manufacturer. After a 24-h transfection, muscle strips were rinsed in warm PBS and placed into a muscle bath for further studies of tonic contraction as described in section of COX-1 and COX-2 siRNA on human GB muscle tonic contraction, COX protein expression, and TxB2 (TXA2 metabolite) and PGE2 production. All muscle strips treated with sense and anti-sense siRNA were examined for expression of the target protein by Western blotting. Transfection experiments were performed in triplicate. Data represent the mean of three different GB specimens.

Transfection efficiency study. To verify the efficiency of the siRNA transfection, parallel control studies were performed by applying a fluorescence labeled oligo (Invitrogen) to muscle strips at the same concentration (100 pmol). After 24 h of incubation, frozen tissue sections (10 µM) were made and optimal transfection efficiency was obtained by use of an Olympus IX50/FIAA fluorescent microscope equipped with a nap-fix camera (Olympus Optical, Melville, NY). The average transfection efficiency in our studies was 65%.

Measurement of tonic contraction in muscle strips. Strips were mounted in 1 ml muscle chambers as described in detail previously (4, 10, 14). Briefly muscle strips were initially stretched to 2.5 g of passive force and equilibrated by continuous perfusion with oxygenated Krebs solution at 37°C. After 1-h perfusion, a basal spontaneous contraction gradually developed and stabilized after another 30-min period of equilibration. Tonic contraction from control muscle strips was maintained stable through the experiment period. Tonic contractions of control and treated muscle strips were measured with Grass isometric force transducers and amplifiers connected to a Biopac data-acquisition system. The viabilities of the muscle strips incubated with culture media, scramble, COX-1, or COX-2 siRNA for 24 h were tested with a maximal concentration (1 µmol) of Ach.

In separate experiments, the effect of mechanical stretch of human muscle strips on tonic contraction, PGE2, and TXA2 production was examined by passively stretching the strips by 1, 2, 3, or 4 g for 1 h and then tonic contraction and PGE2 and TXA2 levels were measured (32, 34).

Western blot. Muscle strips were homogenized in Triton X-100 lyses buffer containing 50 mM Tris·HCl (pH 7.5), 100 mM NaCl, 50 mM NaF, 5 mM EDTA, 1% (vol/vol) Triton X-100, 40 mM β-glycerophosphate, 40 mM 2-mercaptoethanol, 200 µM sodium orthovanadate, 100 µM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, 1 µg/ml pepstatin A, and 1 µg/ml aprotinin. The suspension was centrifuged at 15,000 g for 5 min, and the protein concentration in the supernatant was determined. Then the supernatants were subjected to SDS-PAGE, and the separated proteins were electrophoretically transferred to a nitrocellulose membrane at 30 V overnight. The nitrocellulose membranes were blocked in 5% nonfat dry milk and then incubated with anti-COX-1 or -COX-2 antibodies (1:2,000) for 1-h, followed by 60-min incubation in horseradish peroxidase-conjugated

Downloaded from http://ajpgi.physiology.org/ by 10.220.32.247 on September 8, 2017
secondary antibody (Amersham Biosciences, Piscataway, NJ). Detection is achieved with an enhanced chemiluminescence agent (Amersham Biosciences).

**Measurement of PGE2.** The PGE2 content was measured via a specific PGE2 enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI). As previously described (30, 37, 43), muscle strips or cells were homogenized in homogenization buffer containing 0.1 M phosphate buffer (pH 7.4), 1 mM EDTA, and 20 μg/ml indomethacin at 4°C to inhibit the metabolism of arachidonic acid to prostaglandins. The homogenate was centrifuged at 10,000 g for 15 min at 4°C. The supernatant was used for PGE2 purification using a PGE2-specific affinity column (Cayman). The resulting extracts were dissolved in EIA buffer (1.0 M phosphate buffer, pH 7.4, containing 0.01% NaN₃, 0.037% EDTA, 0.1% BSA). The PGE2 concentration was quantified by using a PGE2 competitive enzyme immunoassay kit and expressed as nanograms per milligram of protein.

**Measurement of TxB2 levels.** TxB2 levels in the muscle cells were measured with a specific EIA kit according to the manufacturer’s instructions (24, 43, 37). Muscle strips were homogenized in eicosanoid homogenization buffer [0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA and 20 μg/ml indomethacin] at 4°C. The homogenate was centrifuged at 12,000 g for 4 min at 4°C and an aliquot of the supernatant was taken for protein measurements. TxB2 (a stable TxA2 metabolite) was assayed in the remaining supernatant by using a TxB2 competitive enzyme immunoassay kit and expressed as nanograms per milligram of protein.

**Statistics.** One- and two-factorial repeated analysis of variance (ANOVA) and Student’s t-tests were used for statistical analysis. P values of <0.05 were considered significant.

**RESULTS**

**Effect of prostaglandins on muscle strip and cell contraction.** The mean basal tonic contraction of guinea pig GB muscle strips was 1.5 ± 0.2 g and of human was 1.98 ± 0.4 g (means ± SE). The mean resting length of intact human GB muscle cells was 59 ± 2 μm and of guinea pig muscle cells was 58.65 ± 1.5 μm. There were also no differences between the lengths and shortening induced by agonists between intact and permeable cells. All three prostaglandins caused contraction of GB muscle strips and cells. Typical tracings show increasing the tonic contraction of GB muscle strips in response to maximal concentrations of TxA2 analog U-46619 and PGE2 (10⁻⁶ M) (Figs. 1A and 2A). Increasing the concentrations of the TxA2 analog U-46619 (Fig. 1, B and C) or PGE2 (Fig. 2, B and C) induced a dose-dependent contraction of muscle strips and dissociated GB smooth muscle cells. The maximal increase in the tonic contraction induced by TxA2 analog U-46619 (10⁻⁷ M) was 2.5 ± 0.3 g and by PGE2 (10⁻⁵ M) was 1.7 ± 0.3 g above basal levels. The maximal cell shortening induced by TxA2 analog U-46619 was 21 ± 1.8% (Fig. 1C) and by PGE2 was 20.6 ± 2% (Fig. 2C). Likewise, dose-response relationship with PGE2 (10⁻⁷ to 10⁻⁶ M) also increased the muscle tension dose dependently with a maximal contraction of 1.7 ± 2 g and cell shortening of 20.5 ± 3% (data not shown).

**Effect of prostaglandin receptor antagonists on GB tonic contraction.** First we examined whether prostaglandins act on G protein-coupled receptors. PGE2-induced contraction is mostly mediated by EP1 receptors because the antagonist against this receptor shifted the dose-response curve to the right (Fig. 3A, P < 0.01, ANOVA). This EP1 receptor antagonist SC-19220 had no effect on suprostone, an EP3 receptor agonist. The Gq/11 protein antibody (1:400 titer) blocked the agonist SC-19220 had no effect on suprostone, an EP3 receptor agonist. The Gq/11 protein antibody (1:400 titer) blocked the increase in [³⁵S]-GTPγS binding to Gq/11 protein induced by PGE2 (Fig. 3C, P < 0.001, Student’s t-test). Thus PGE2 appears to activate G protein-
coupled receptors because its EP1 receptor couples to Gq/11 protein. Second, studies were performed to examine whether prostaglandins contribute to the maintenance of basal tonic contraction by acting on these membrane receptors. Figure 4A illustrates the inhibitory actions of the TxA2 receptor antagonist SQ-29548. Moreover, as shown in Fig. 4B, GB muscle strips were treated with buffer alone (controls) or with increas-

Fig. 2. A: typical tracing illustrates a GB muscle strip tonic contraction and contraction in response to a maximal concentration of PGE2 (10^{-5} M). Contraction of muscle strips (B) and dissociated cells (C) in response to increasing concentrations of PGE2. Values are means ± SE of 3 guinea pig GB.

Fig. 3. A: effect of a maximal concentration (10^{-5} M) of SC-19220 (EP1 receptor antagonist) on the contraction induced by increasing concentrations of PGE2. The receptor antagonist shifted the dose-response relationship to the right (P < 0.01 by ANOVA). Values are means ± SE of 3 experiments. B: Gq/11 protein antibody (Ab; titer 1:400) blocked the contraction induced by increasing concentrations of PGE2 (P < 0.001 by ANOVA). C: PGE2 increased the 35S-GTPγS binding to Gq/11 of up to 64 ± 6% over basal levels, which was reduced to 17 ± 3% by pretreatment with the EP-1 receptor antagonist SC-19220 (10^{-5} M) (P < 0.001, unpaired Student’s t-test). Values are means ± SE of 3 guinea pig GB.
ing concentrations of SC-19220 (an EP1 receptor antagonist) or with SQ-29548 (a TxA2 receptor antagonist). The basal tonic contraction was reduced dose dependently by either antagonist compared with buffer alone, which remained stable throughout the study ($P < 0.01$ per ANOVA). The maximal reduction of the tonic contraction induced by SC-19220 at the concentration of $10^{-6}$ M was $56.3 \pm 0.4\%$ ($P < 0.01$, per ANOVA). This concentration of SC-19220 also partially blocked the contraction induced by a maximal concentration of PGE2 (Fig. 3A). Similar results were found when the muscle strips were treated with the TxA2 receptor antagonist SQ-29548 ($P < 0.01$, ANOVA). The maximal reduction in muscle strip tonic contraction induced by SQ-29548 was $65.8 \pm 1\%$ at the concentration of $10^{-6}$ M. Even though PGF2a caused a dose-dependent contraction ($P < 0.005$ per ANOVA), its receptor antagonist AI-8810 did not inhibit the basal GB tonic contraction at the maximal concentration of $10^{-6}$ M.

**Effect of COX inhibitors on GB tonic contraction.** Selective COX inhibitors at concentrations of $10^{-6}$ M partially lowered the tonic gallbladder contraction (Fig. 5). The COX-1 inhibitor SC-560 partially reduced the tonic contraction from $2.19 \pm 0.1$ to $0.77 \pm 0.06$ g ($P < 0.01$, unpaired Student’s $t$-test). The COX-2 inhibitor NS-398 also partially reduced the tonic contraction from $2.19 \pm 0.1$ to $0.94 \pm 0.11$ g ($P < 0.01$, unpaired Student’s $t$-test). The tonic contraction was almost completely abolished when the muscle strips were treated with both inhibitors (Fig. 5, $P < 0.001$). SC-560 also decreased TxB2 levels (TxA2 stable metabolite) without impairing PGE2 levels (Fig. 6B, $P < 0.001$ unpaired Student’s $t$-test), whereas NS-398 lowered the PGE2 levels significantly (Fig. 6A, $P < 0.001$) without affecting TxB2 levels of the gallbladder muscle. Moreover, muscle strips were treated with increasing concentrations of indomethacin from $10^{-9}$ to $10^{-6}$ M or with buffer alone (controls) for 10 min (Fig. 7A). The basal tonic tension was

![Fig. 4. A: tracing illustrates the inhibitory effect of TxA2 receptor antagonist SQ-29548 on gallbladder tonic contraction. B: effect of increasing concentrations of SQ-29548 and of SC-19220 (EP1 receptor antagonist) on GB tonic contraction. Both antagonists partially decreased the tonic contraction ($P < 0.01$ per ANOVA). Values are means ± SE of 3 guinea pig GB.](image1)

![Fig. 5. Effect of cyclooxygenase (COX)-1 inhibitor SC-560 and COX-2 inhibitor NS-398 (10^{-6} M) on basal GB tonic contraction. Each inhibitor reduced the GB tonic contraction partially ($^*P < 0.01$, unpaired Student’s $t$-test). The tonic contraction was almost completely blocked by the administration of both inhibitors ($P < 0.001$, unpaired Student’s $t$-test). Values are means ± SE of 3 guinea pig GB.](image2)

![Fig. 6. Effect of COX-1 inhibitor SC-560 and COX-2 inhibitor NS-398 (10^{-6} M) on PGE2 and TxB2 levels in GB muscle strips. A: COX-2 inhibitor reduced PGE2 levels ($^*P < 0.001$, Student’s $t$-test) without affecting TxB2 levels. B: COX-1 inhibitor caused a decrease in TxB2 levels ($^*P < 0.001$, unpaired Student’s $t$-test) but had no effect on PGE2 levels. Values are means ± SE of 3 guinea pig GB.](image3)
significantly lowered dose dependently by indomethacin. Indomethacin 10^{-9} \text{ M} almost completely abolished the tonic contraction (P < 0.001 compared with control per ANOVA). B: indomethacin-induced inhibition of the tonic contraction (P<0.001) was restored by PGE2 at a concentration of 10^{-6} \text{ M} (P<0.001). Values are means ± SEM of 3 guinea pig GB.

Fig. 7. Effect of increasing the concentrations of the nonselective COX inhibitor indomethacin (Indo; 10^{-9} to 10^{-6} \text{ M}) on GB tonic contraction (A). Indomethacin dose dependently inhibited the GB tonic contraction (P<0.001 compared with control per ANOVA). B: indomethacin-induced inhibition of the tonic contraction (P<0.001) was restored by PGE2 at a concentration of 10^{-6} \text{ M} (P<0.001). Values are means ± SEM of 3 guinea pig GB.

COX-1 or COX-2 siRNA transfection reduced the tonic contraction of muscle strips compared to control (Fig. 8A, P < 0.01, Student’s t-test). However, the COX-1 or COX-2 siRNA transfected strips contracted in response to ACh with a delta contraction that was not different from those of strips transfected with scramble siRNA or only with tissue culture medium (Fig. 8B). COX-1 enzyme expression was reduced by COX-1 siRNA without affecting COX-2 protein expression (Fig. 9A, P < 0.01, unpaired Student’s t-test) compared with scramble siRNA or tissue culture treatment, whereas transfection with COX-2 siRNA lowered the expression of COX-2 protein without altering the levels of COX-1 protein levels (Fig. 9B; P < 0.01).

Moreover, COX-1 or COX-2 siRNA transfection that lowered the expression of COX enzymes and GB tension was accompanied by changes in the levels of PGE2 and TXB2. COX-1 siRNA treatment decreased TXB2 synthesis (P<0.01) but it did not alter the levels of PGE2 (Fig. 10B). In contrast, COX-2 siRNA transfection significantly reduced PGE2 levels (P<0.01) but the TXB2 levels remain unchanged (Fig. 10A).
Effect of stretching muscle strip on active tonic contraction and prostaglandins. The role of COX enzymes and prostaglandins on changes in GB active tonic contraction was examined by stretching GB muscle strips (32, 34) by 1, 2, 3, and 4 g. Increases in passive stretching lead to a length-dependent increase in active tonic contraction and in PGE2 and TXB2 levels. Tonic contraction was determined 1 h after the strips had been stretched. There was a length-dependent increase in active tension with the highest after stretching the strips by 4 g (Fig. 11). The expected rise in tonic contraction was blocked almost completely by treatment with indomethacin before stretching the strips (P < 0.001, ANOVA) and also inhibited the expected rise of TXB2 and of PGE2 levels (Fig. 12, A and B; P < 0.001, ANOVA). Pretreatment with COX-1 or COX-2 inhibitors partially inhibited the expected increase in active tension (Fig. 11; P < 0.01, ANOVA). COX-1 inhibitor SC-560 blocked the rise of TXB2 (Fig. 12, A and B) without affecting PGE2 levels. The COX-2 inhibitor NS-398 also partially inhibited the expected increase in PGE2 levels without impairing the rise of TXB2 levels.

DISCUSSION

We have previously shown that the tonic contraction of human and guinea pig GB, although regulated by neurohor-
monal inputs, is mostly generated by myogenic mechanisms because it is unaffected by functional denervation with TTX (3, 12). The present data showed that there is a reciprocal dependency between alterations of PGE2 and TxA2 levels and changes in tonic contraction. PGE2 is able to generate the tonic contraction by continuously stimulating their receptors because their desensitization period is extremely short (2, 19, 26, 31, 50).

Both prostaglandins contract GB muscle strips and cells dose dependently by acting on specific G protein-coupled receptors because their actions are blocked by receptor antagonists and by antibodies against Gq/11 proteins. Although it is well established that TxA2 contracts most muscle cells that have been studied (7, 19–21), PGE2 contracts or relaxes gastrointestinal muscle cells depending on the population of receptors present in these cells. EP1 and EP3 receptor mediate contraction, whereas EP2 and EP4 mediate relaxation (1, 18). PGE2 relaxes muscle cells in the circular muscle layer, but it contracts muscle cells in the longitudinal layer of the gastrointestinal tract (47).

Moreover, the EP1 and TP receptors appear to be the main receptors that mediate the actions of endogenous prostaglandins that contribute to the maintenance of GB tonic contraction. Their respective receptor antagonists dose dependently reduced the GB tonic contraction. However, the role of EP3 receptor in partially mediating the actions of PGE2 cannot be ruled out because its agonist suprostil also contract GB muscle strips.

These findings are also supported by the actions of nonselective and selective inhibitors of COX enzymes that decrease the tonic contraction and reduce the levels of PGE2 and TxA2. Both COX-1 and COX-2 appear to be constitutive enzymes in GB muscle cells generating basal levels of both TxA2 and PGE2, respectively. These findings are in agreement with previous observations in the muscularis propria of the murine proximal colon that COX-2 can also be constitutive (36).

COX-1 enzyme appears to generate TxA2 whereas PGE2 is dependent on COX-2. Inhibition of COX-1 lowers TxA2 levels and transfection of muscle strips with COX-1 siRNA reduce the expression of COX-1 and production of TxA2, whereas a selective inhibitor of COX-2 reduces PGE2 synthesis. The specific inhibition and reduced expression of each enzyme by siRNA transfection resulted in partial reduction of tonic contraction, suggesting that both prostaglandins contribute to the genesis of GB tonic contraction. Our findings also are in agreement with previous studies showing that COX-1 and COX-2 biosynthetic pathways are segregated within cells (41) and utilize different pools of arachidonic acid (28). COX-2 couples preferentially with PGE synthase to generate PGE2 and requires lower concentrations of arachidonic acid. Furthermore, specific COX-2 inhibitors do not affect thromboxane levels. In contrast, COX-1 couples with TxA2 synthase and requires higher arachidonic concentrations (41). The tonic contraction was abolished after treatment with both selective COX inhibitors or by the nonselective inhibitor

Fig. 12. PGE2 and TXB2 levels in TTX-treated human GB muscle strips (devoid of mucosa and serosa) without stretch (control) and muscle strips stretched by increasing the tension by 1, 2, 3, and 4 g. A: increase in PGE2 levels was inhibited by pretreatment with indomethacin (10^{-5} M) or with NS-398 (10^{-6} M). The latter did not affect the increases in TXB2. *P < 0.001 by ANOVA. B: pretreatment of muscle strips with indomethacin or with the COX-1 inhibitor SC-560 (10^{-6} M) blocked the increases in TXB2 levels (*P < 0.001 compares buffer with indomethacin, ~P < 0.001 compares buffer with SC-560 treatment, ANOVA), whereas the COX-2 inhibitor SC-560 (10^{-6} M) had no effect. Values are means ± SE of 3 human GB.
indomethacin. Indomethacin does not affect calcium influx or calcium release from the intracellular stores because this inhibitor does not block the contraction induced by PGE2, ACh, or CCK-8 (27).

Mechanical stretching of human GB muscle strips caused a length-dependent increase in active tonic contraction and a concomitant increase in the levels of TxB2 (the TxA2 metabolite) and PGE2. Nonselective and selective inhibitors of COX enzymes prevented the increase in tonic contraction induced by muscle stretching and the expected increase in TxB2 levels when pretreated with COX-1 inhibitor and in PGE2 levels when pretreated with COX-2 inhibitor. The increase in prostaglandins in response to mechanical stretch is consistent with studies in myometrial cells (42). Passive stretching muscle strip resulted in an increase in COX-2 protein and PGE2 concentrations (34).

In summary, these studies show that both TxA2 and PGE2 contribute to the genesis of GB tonic contraction in the guinea pig and human GB muscle cells by functioning as a local hormones or autacoids. These muscle cells synthesize prostaglandins and export them into the extracellular space, where they act on G protein-coupled receptors, which are mostly EP1 and TP receptors (19, 23, 45). Receptor antagonists blocked the excitatory actions of both PGE2 and TxA2, and Gq/11 antibodies blocked PGE2-induced contraction. COX-1 and COX-2 enzymes are constitutive, generating basal levels of TxA2 and PGE2, respectively. The enzyme inhibition or lower expression were accompanied by parallel changes in TxA2 and PGE2.

In studies supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant RO1-DK027389 –20A.

REFERENCES


