Gastrin inhibits secretin-induced ductal secretion by interaction with specific receptors on rat cholangiocytes

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Gastrin inhibits secretin-induced ductal secretion by interaction with specific receptors on rat cholangiocytes. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1061–G1070, 1997.—We assessed the effect of gastrin on ductal secretion in normal and bile duct-ligated (BDL) rats. The effect of gastrin on ductal secretion was examined in the presence of proglumide, a specific antagonist for gastrin receptor (GR). We isolated pure cholangiocytes from normal and BDL rats and assessed gastrin effects on secretin receptor (SR) gene expression and intracellular adenosine 3',5'-cyclic monophosphate (cAMP) levels. We examined the presence of GR mRNA in cholangiocytes by reverse transcription polymerase chain reaction (RT-PCR). In normal or BDL rats, gastrin produced no changes in spontaneous bile secretion. Simultaneous infusion of gastrin inhibited secretin-induced cholestasis and bicarbonate output in BDL rats. In the presence of proglumide gastrin did not inhibit secretin-induced cholestasis in BDL rats. Gastrin decreased in cholangiocytes from BDL rats 1) SR gene expression and 2) secretin-induced cAMP levels. With the use of RT-PCR, GR mRNA was detected in cholangiocytes. Similar to what is shown for secretin and somatostatin, we propose that the opposing effects of secretin and gastrin on cholangiocyte secretory activity regulate ductal secretion in rats.

bile duct ligation; biliary epithelium; hormones; proglumide; adenosine 3',5'-cyclic monophosphate
GASTRIN MODULATION OF SECRETIN-INDUCED DUCTAL SECRETION

MATERIALS AND METHODS

Animal model. Male Fischer 344 rats (150 to 200 g) were purchased from Charles River (Wilmington, MA). The animals were maintained in a temperature-controlled environment (20 ± 2°C) with a 12:12-h light-dark cycle and had free access to standard rat chow and water before each experiment. The studies were conducted in normal rats and in rats with cholangiocyte proliferation induced by BDL for 14 days (1, 3). BDL was performed as previously described (1, 3, 7, 53). Before each experiment, animals were anesthetized with pentobarbital sodium (50 mg/kg ip). Study protocols were performed in compliance with the institutional guidelines.

Materials. Reagents were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated. Secretin was purchased from Peninsula Laboratories (Belmont, CA). The mouse anti-cytokeratin 19 (CK-19) antibody was purchased from Amersham (Arlington Heights, IL). Radioimmunoassay (RIA) kits for the determination of intracellular cAMP levels in cholangiocytes from normal and BDL rats were purchased from Amersham.

In situ immunohistochemistry. The number of intrahepatic bile ducts present in frozen liver sections (6-μm thick) randomly obtained from normal and BDL rats was determined by immunohistochemistry for CK-19 (2, 31), a specific marker for cholangiocytes in rat liver (2, 4, 5). Only two to three intrahepatic bile ducts (stained for CK-19) were present within portal areas of frozen sections obtained from normal rat liver (results not shown). After BDL there was a marked increase in the number of intrahepatic bile ducts limited to portal areas (results not shown). These results are in agreement with a number of reports (1, 2, 53).

Isolation and phenotypic characterization of cholangiocyte preparations from normal and BDL rat livers. After standard collagenase perfusion, a mixed nonparenchymal cell fraction (40–50% pure by γ-glutamyl transpeptidase) (7, 25) was obtained from intact portal tracts of both normal and BDL rat liver and further purified by immunoaffinity separation (5, 7, 25, 31, 53) using a monoclonal antibody expressed by all intrahepatic cholangiocytes (25). By this approach, virtually pure preparations of cholangiocytes were obtained from both normal and BDL rat livers. Cell number and viability (≥99%) were assessed by trypan blue exclusion.

In vivo studies of biliary physiology. Before being used for in vivo studies of biliary physiology, rats were fed ad libitum. After anesthesia with pentobarbital sodium (50 mg/kg ip), both normal and BDL rats were surgically prepared for bile collection as previously described (1, 3, 31, 53). Briefly, the jugular vein was cannulated with a PE-50 cannula (Clay-Adams, New York, NY) to infuse either Krebs-Henseleit bicarbonate solution (KRH), gastrin, gastrin plus secretin, or secretin alone dissolved in KRH (see below). Blood was withdrawn every 10 min from one carotid artery to assess the arterial hematocrit, which remained constant (41–45%) in all animals during bile collection. The rate of fluid infusion was adjusted according to both the rate of bile flow and the value of the arterial hematocrit and ranged from 0.738 to 2.964 ml/h. Body temperature was monitored with a rectal thermometer (Yellow Springs Instruments, Yellow Springs, OH) and maintained at 37°C with a heating pad. When steady-state bile flow was achieved (60–70 min from the beginning of bile collection), we infused gastrin (10⁻⁷ M) for 30 min, then KRH for 60 min followed by simultaneous infusion of gastrin (10⁻⁷ M) and secretin (10⁻⁷ M) for 30 min, then KRH for 60 min followed by infusion of secretin (10⁻⁷ M) for 30 min and a final infusion of KRH for 60 min. In a separate set of experiments we also determined in BDL rats the in vivo dose-dependent effects of gastrin (10⁻⁷, 10⁻⁸ M) on both secretin-induced choleretics and bicarbonate output.

The effect of gastrin on both spontaneous and secretin-induced bile flow and bicarbonate output was also examined in the presence of proglumide [N-(benzoyl)-L-glutamic acid-1-di-n-propylamide] (75 mg·kg body wt⁻¹·h⁻¹), a specific antagonist for the GR (26, 59, 60). The dose of gastrin used in the present studies was similar to that of other reports (26). When spontaneous steady-state bile flow was achieved (60–70 min from the beginning of bile collection), proglumide was infused until steady-state bile flow was once more achieved. Then, in the presence of proglumide, gastrin (10⁻⁷ M) and secretin (10⁻⁷ M) were infused together for 30 min, followed by a final infusion of KRH for 60 min. At the end of each experiment, the animal was euthanized with an overdose of sedative (200 mg/kg ip) and the liver was removed and weighed. During each experiment, bile was collected every 10 min in preweighed tubes and immediately stored at −20°C before determining bicarbonate concentration. Bile volume was determined by weight, assuming a density of 1.0 g/ml. Bile flow was expressed as microliters per minute per kilogram body weight. Bicarbonate concentration (measured as total CO₂) in bile from control and BDL rats was determined by a Natelson microgasometer apparatus (Scientific Industries, Bohemia, NY).

Molecular localization of GRs on cholangiocytes from normal and BDL rats. To determine if the effects of gastrin on secretin-induced ductal bile secretion occur by interaction with specific receptors on cholangiocytes, we examined the genetic expression of GR mRNA by RT-PCR using cholangiocyte poly(A)⁺ mRNA obtained from normal and BDL rat liver. To examine for the presence of GR mRNA in cholangiocytes we used specific primers (sense 5’-TGTCACAACCTCCG-GTTCC-3’ and antisense 5’-GCTGATGGTGTTAGTATGC- TAGCC-3’), expected fragment length 444 bp) designed from the sequence encoding for the rat GR gene (28). Poly(A)⁺ mRNA from rat brain and yeast tRNA were the positive and negative controls, respectively, for the GR gene. The comparability of the total messenger RNA used was assessed by RT-PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the housekeeping gene (5, 7). Primers for GAPDH were based on the rat GAPDH sequence (sense 5’-GACTTCAACGAGACTCCATTCTC-3’ and antisense 5’-TATGGGTCTGGGATGGAATTGTG-3’). The following RT-PCR conditions were used: 5 ng of poly(A)⁺ mRNA (35 step cycles: 30 s at 94°C, 30 s at 58°C, and 45 s at 72°C). To confirm the identity, the PCR fragments were sequenced using a Sequenase version 2.0 kit (United States Biochemical, Cleveland, OH). Poly(A)⁺ mRNA was extracted from isolated cholangiocytes (2 × 10⁶) and tissues (100 mg) by the Micro-Fast Track II kit (Invitrogen, San Diego, CA) (7) according to the instructions supplied by the vendor. The relative intensity of the autoradiograms was determined by densitometry.

In vitro effect of gastrin on SR gene expression. After isolation, pure preparations of cholangiocytes (≈2.0 × 10⁵) from both normal and BDL rat liver were treated in the presence of 0.2% bovine serum albumin (BSA) for 15 min at 37°C with gastrin (10⁻⁷ M), secretin (10⁻⁷ M), gastrin plus secretin (both at 10⁻⁷ M), or 0.2% BSA (control) and subse-
sequently analyzed for the genetic expression of the SR gene. We used rat heart and rat kidney RNA as positive and negative controls, respectively, for the SR gene. The equality of the RNA used was assessed by hybridization with the housekeeping gene GAPDH (5, 7). Rat kidney and yeast tRNA were the positive and negative controls, respectively, for the GAPDH gene. The expression of selected messages was determined by lysate ribonuclease protection assay (Direct Protect, Amblion, Austin, TX) (31) according to the instructions of the vendor; each cell lysate sample contained 4.50 × 10^6 pure cholangiocytes obtained from normal or BDL rat liver. This approach has been previously used by us (31) to exactly quantify SR gene expression among cholangiocytes isolated from normal and partial hepatectomized rat livers.

Antisense riboprobes were transcribed from linearized cDNA templates with T7 or SP6 RNA polymerase using [α-32P]UTP (800 Ci/mmol) (Amersham). The following [32P]UTP-labeled single-stranded antisense riboprobes were used: a 316-bp riboprobe encoding a complementary sequence for rat GAPDH mRNA was obtained from cDNA purchased from Ambion and a 319-bp riboprobe encoding the message for the SR gene transcribed from a P-element-derived transgene (gift of Dr. N. LaRusso, Rochester, MN) (7, 31).

Intracellular cAMP levels. Both spontaneous and hormone-induced intracellular cAMP levels were measured in isolated cholangiocytes as previously described by us and others (5, 6, 27, 30, 31, 41, 53). After purification, pure preparations of cholangiocytes (1.0 × 10^6) were incubated for 1 h at 37°C to regenerate membrane proteins damaged by treatment with proteolytic enzymes (6, 27, 31, 48) and subsequently stimulated in the presence of 0.2% BSA with gastrin (10^{-7} M), secretin (10^{-7} M), gastrin plus secretin (both at 10^{-7} M), or BSA for 5 min at 22°C, a temperature at which cAMP synthesis is commonly measured (5, 8, 27, 30, 31, 41, 53). Before determination of intracellular cAMP levels, a phosphodiesterase inhibitor, 0.5 mM 3-isobutyryl-methylxanthine, was added to all cholangiocyte preparations used (5, 8, 27, 30, 31, 41, 53). After ethanol extraction, spontaneous and agonist-induced cAMP formation was measured by RIA using commercial kits (Amersham) according to the instructions supplied by the vendor.

Statistical analysis. All data are expressed as means ± SE. The differences between groups were analyzed by Student’s t-test when two groups were analyzed or analysis of variance (ANOVA) if more than two groups were analyzed.

RESULTS

In vivo studies of biliary physiology. In normal rat liver, spontaneous bile flow (80.75 ± 6.69 µl·min^{-1}·kg body wt^{-1}) and basal bicarbonate output (2.37 ± 0.15 µeq·min^{-1}·kg body wt^{-1}) were similar to that of previous studies (1, 3, 30, 31, 53) (see Fig. 1, A and B). As expected (1, 3, 30, 31, 53), in normal rats secretin did not alter ductal bile secretion (75.72 ± 9.00 µl·min^{-1}·kg body wt^{-1}) or bicarbonate output (2.41 ± 0.16 µeq·min^{-1}·kg body wt^{-1}) compared with their corresponding basal values (80.75 ± 6.69 µl·min^{-1}·kg body wt^{-1} and 2.37 ± 0.15 µeq·min^{-1}·kg body wt^{-1}, respectively, see above) (Fig. 1, A and B). In normal rats, intravenous infusion of gastrin (10^{-7} M), alone or in combination with secretin (10^{-7} M), did not induce changes in bile secretion or bicarbonate output (see Fig. 1, A and B).

In agreement with a number of reports (1, 3, 30, 31, 53), after BDL there was a significant increase in both spontaneous bile secretion (99.88 ± 4.65 µl·min^{-1}·kg body wt^{-1}, P < 0.05) and bicarbonate output (3.48 ± 0.20 µeq·min^{-1}·kg body wt^{-1}, P < 0.05) compared with normal control rats (80.75 ± 6.69 µl·min^{-1}·kg body wt^{-1} and 2.37 ± 0.15 µeq·min^{-1}·kg body wt^{-1}, respectively) (Fig. 1, A and B). As expected (1, 3, 30, 31, 53), in BDL rats secretin induced a massive increase in both bile secretion (+79.37 ± 9.19 µl·min^{-1}·kg body wt^{-1}, P < 0.05 vs. its corresponding basal value) and bicarbonate output (+7.24 ± 1.15 µeq·min^{-1}·kg body wt^{-1} vs. its corresponding basal value, P < 0.05) (Fig. 1, A and B). In BDL rats, gastrin alone (10^{-7} M) did not cause significant changes in bile secretion (106.75 ± 6.01 vs. 99.88 ± 4.65 µl·min^{-1}·kg body wt^{-1} vs. its corresponding basal value) or bicarbonate output (3.19 ± 0.30 vs. 3.48 ± 0.20 µeq·min^{-1}·kg body wt^{-1} vs. its corresponding basal value) (Fig. 1, A and B). The dose-response curve shows that gastrin, similar to

Fig. 1. Effect of secretin and gastrin on bile secretion (A) and biliary bicarbonate output (B) in both normal and bile duct-ligated (BDL) rats. A: selected hormones (10^{-7} M) were infused for 30 min via a jugular vein cannula after a 60-min equilibration period with Krebs-Henseleit bicarbonate solution (KRH). Bile was collected every 10 min and expressed as microliters per minute per kilogram body weight. *P < 0.05 vs. its basal value; **P < 0.05 vs. secretin-stimulated bile secretion. B: bicarbonate concentration (measured as total CO2) in bile from normal and BDL rats was determined by a Nalson microgasometer apparatus. Data were expressed as microequivalents per minute per kilogram body weight. *P < 0.05 vs. its corresponding value from normal rat liver; **P < 0.05 vs. its basal value; **P < 0.05 compared with secretin-stimulated bicarbonate output. Data are means ± SE for 5 rats. Statistical analysis was performed by both analysis of variance (ANOVA) and unpaired t-test. Note that scales on vertical axes are different.
what was observed at $10^{-7}$ M (Fig. 1A and B), did not alter significantly bile secretion or bicarbonate output when infused in vivo in BDL rats at the concentration of $10^{-10}$ M ($+9.81 \pm 5.59 \mu$eq·min$^{-1}$·kg body wt$^{-1}$ and $+0.069 \pm 0.21 \mu$eq·min$^{-1}$·kg body wt$^{-1}$, respectively, vs. its corresponding basal value), $10^{-9}$M ($+1.97 \pm 5.09 \mu$eq·min$^{-1}$·kg body wt$^{-1}$ and $+0.33 \pm 0.14 \mu$eq·min$^{-1}$·kg body wt$^{-1}$, respectively, vs. its corresponding basal value), or $10^{-8}$ M ($+0.97 \pm 3.10 \mu$eq·min$^{-1}$·kg body wt$^{-1}$ and $+0.27 \pm 0.33 \mu$eq·min$^{-1}$·kg body wt$^{-1}$, respectively, vs. its corresponding basal value). In contrast, simultaneous infusion of gastrin and secretin (both at $10^{-7}$ M) markedly decreased secretin-induced cholereticosis (133.26 $\pm$ 9.34 vs. 195.78 $\pm$ 22.88 µl·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) and biliary bicarbonate output (5.41 $\pm$ 0.59 vs. 10.72 $\pm$ 1.11 µeq·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) in BDL rats (see Fig. 1A and B). In a fashion similar to that observed at the concentration of $10^{-7}$ M (Fig. 1A), gastrin also inhibited the choleric effect of secretin (i.e., $+79.37 \pm 9.19$ µeq·min$^{-1}$·kg body wt$^{-1}$; Fig. 2A) at both $10^{-8}$ M ($+22.83 \pm 5.85$ vs. $+79.37 \pm 9.19$ µeq·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) and $10^{-9}$ M ($+35.31 \pm 10.95$ vs. $+79.37 \pm 9.19$ µeq·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) (Fig. 2A). Similarly, gastrin markedly decreased secretin-induced bicarbonate output (i.e., $+7.24 \pm 1.15$ µeq·min$^{-1}$·kg body wt$^{-1}$; Fig. 2B) at both $10^{-8}$ M ($+1.49 \pm 0.45$ vs. $+7.24 \pm 1.15$ µeq·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) and $10^{-9}$ M ($+1.63 \pm 0.71$ vs. $+7.24 \pm 1.15$ µeq·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) (Fig. 2B).

Consistent with the concept that specific GRs are present in cholangiocytes, simultaneous infusion of gastrin ($10^{-7}$ M) and proglumide (75 mg·kg body wt$^{-1}$·h$^{-1}$) did not inhibit secretin-induced cholereticosis (251.69 $\pm$ 15.38 vs. 149.88 $\pm$ 8.52, basal value, P $<$ 0.05; Fig. 3B) or secretin-stimulated bicarbonate output (14.21 $\pm$ 1.17 vs. 4.78 $\pm$ 0.07, basal value, P $<$ 0.05; Fig. 3C) typical of BDL. As expected, proglumide did not alter the effect of gastrin and secretin on bile flow or bicarbonate excretion in normal rats (Fig. 3A, 3B, and C, respectively). In agreement with previous reports (50), proglumide caused an increase in bile flow in both normal (175.52 $\pm$ 12.16 vs. 85.77 $\pm$ 10.98 µl·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) and BDL (144.00 $\pm$ 6.02 vs. 109.00 $\pm$ 9.86 µl·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) rats (see Fig. 3A and B). Similarly, proglumide increased biliary bicarbonate excretion in both normal (5.74 $\pm$ 0.34 vs. 2.27 $\pm$ 0.21 µeq·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) and BDL (5.08 $\pm$ 0.36 vs. 3.24 $\pm$ 0.37 µeq·min$^{-1}$·kg body wt$^{-1}$, P $<$ 0.05) rats (Fig. 3C).

Localization of GRs on cholangiocytes. As shown in Fig. 4 by RT-PCR a 444-bp product was detected in cholangiocytes from both normal and BDL rat livers. Sequence analysis of the PCR fragment for GR mRNA showed 100% homology to the published sequence for the GR gene (28). In a fashion similar to that observed for other genes (e.g., SR and SRTR2), the expression of GR mRNA was greater (=1.5-fold) in isolated cholangiocytes from BDL rats compared with normal cholangiocytes (Fig. 4B). The expression of GAPDH mRNA [the housekeeping gene (5, 7, 8)] was similar between normal and proliferating cholangiocytes in agreement with our previous studies (5, 7, 8, 53).

Effect of gastrin on SR gene expression. To begin to investigate the mechanisms by which gastrin, in concert with secretin, modulates ductal secretory activity we studied, in cholangiocytes from both normal and BDL rats, the effect of gastrin on the genetic expression of SR, an important marker of ductal bile secretion (4, 5, 7, 31). The genetic expression of GAPDH, the housekeeping gene used to assess the comparability of the RNA used (5, 7, 8), was similar among basal and hormone-stimulated cholangiocytes (Fig. 5A). As shown in Fig. 5A, a 318-bp transcript for the SR gene was present in heart RNA (positive control) (5, 7, 8, 31). In agreement with our previous studies (5, 7, 8, 31), SR
Gene expression was detected in normal cholangiocytes (Fig. 5, A and B). In normal cholangiocytes, gastrin, secretin, or gastrin plus secretin (all at 10^{-7} M) had no effect on SR gene expression (Fig. 5, A and B). After BDL, there was an approximately twofold increase in SR gene expression, a finding that parallels our previous studies (7) (Fig. 5, A and B). In cholangiocytes from BDL rats, secretin (10^{-2} M) increases (>70%) SR gene expression (Fig. 5, A and B) that parallels the increase in ductal bile secretion observed during in vivo secretin infusion (Fig. 1A). In a fashion similar to that observed for ductal bile flow (Fig. 1), gastrin (in combination with secretin) decreases (<50%) the genetic expression of SR in isolated cholangiocytes from BDL rat livers (Fig. 5, A and B). Gastrin alone did not affect SR gene expression (Fig. 5, A and B). Densitometric data are the means of two experiments.

Basal and hormone-regulated intracellular cAMP levels. In normal cholangiocytes, basal intracellular cAMP levels (18.71 ± 2.64 fmol/100,000 cells) were similar to that reported in previous studies by us (31) and others (27) (Fig. 6). Spontaneous intracellular cAMP levels markedly increased in cholangiocytes from BDL rats compared with normal cholangiocytes (28.98 ± 1.30 vs. 18.71 ± 2.64 fmol/100,000 cells, P < 0.05) (Fig. 6). As expected, secretin (10^{-7} M) markedly increased intracellular cAMP levels in both normal (35.00 ± 3.93 fmol/100,000 cells, P < 0.05 vs. basal value) and hyperplastic (60.17 ± 3.36 fmol/100,000 cells, P < 0.05 vs. basal value) cholangiocytes (Fig. 6).
Gastrin (10^-7 M) alone did not affect basal intracellular cAMP levels in cholangiocytes from normal or BDL rats (see Fig. 6), but markedly inhibited secretin-induced increases in cAMP levels in both normal (17.65 ± 0.43 vs. 35.00 ± 3.93 fmol/100,000 cells, P < 0.05) and proliferating cholangiocytes (19.40 ± 0.36 vs. 60.17 ± 3.36 fmol/100,000 cells, P < 0.05) (Fig. 6). The data closely parallel the inhibitory effect of gastrin on bile secretion (Figs. 1 and 2) and SR gene expression (Fig. 5).

Similar to that observed with 10^-7 M gastrin (Fig. 6), dose-response analysis shows that gastrin did not affect intracellular cAMP synthesis in hyperplastic cholangiocytes at the concentration of 10^-8 (23.40 ± 2.80 vs. 28.98 ± 1.30 fmol/100,000 cells, basal value), 10^-9 (24.56 ± 5.35 vs. 28.98 ± 1.30 fmol/100,000 cells, basal value), or 10^-10 M (16.72 ± 6.26 vs. 28.98 ± 1.30 fmol/100,000 cells, basal value). Parallel with the changes in secretin-induced cholestasis observed after gastrin administration (Figs. 1 and 2), gastrin markedly decreased secretin-induced cAMP synthesis in proliferating cholangiocytes at the concentration of 10^-8 (15.71 ± 3.25 vs. 60.17 ± 3.36 fmol/100,000 cells, P < 0.05), 10^-9 (23.98 ± 4.75 vs. 60.17 ± 3.36 fmol/100,000 cells, P < 0.05), and 10^-10 M (17.35 ± 0.85 vs. 60.17 ± 3.36 fmol/100,000 cells, P < 0.05) (Fig. 7).

**DISCUSSION**

These novel studies demonstrated the role of gastrin in the regulation of secretin-stimulated ductal bile secretion in BDL rat liver. In normal rats, in vivo infusion of gastrin at 10^-7 M, alone or in combination with secretin (10^-7 M), produced no significant changes in bile secretion or biliary bicarbonate output. In BDL rats, gastrin alone (10^-7-10^-10 M) produced no significant changes in bile secretion or bicarbonate output. In contrast, simultaneous infusion of gastrin (10^-7-10^-9 M) plus secretin (10^-7 M) markedly decreased both secretin-induced choleresis and biliary bicarbonate output in BDL rats. Gastrin, when infused simultaneously with proglumide [a specific inhibitor for the GR, (26, 59, 60)], did not inhibit secretin-induced choleresis or secretin-stimulated bicarbonate output in BDL rats. In isolated cholangiocytes, we have shown that gastrin decreased SR gene expression and secretin-induced cAMP synthesis. Finally, we have detected GR mRNA in cholangiocytes isolated from both normal and BDL rats.
cholangiocytes were incubated at 37°C for 1 h (to restore membrane proteins damaged by enzymatic digestion) and then stimulated at 22°C for 5 min in the presence of 0.2% BSA with Proteins damaged by enzymatic digestion) and then stimulated at 22°C for 5 min in the presence of 0.2% BSA with gastrin plus secretin (both 10⁻⁷ M); 3) secretin (10⁻⁷ M); or 4) 0.2% BSA (control). **P < 0.05 vs. its corresponding value from normal rat liver. **Hormone-induced cAMP levels of cholangiocytes differ significantly from their corresponding basal levels (P < 0.05). *Gastrin-modulated cAMP synthesis of cholangiocytes differs significantly from secretin-stimulated cAMP levels (P < 0.05). Data are means ± SE for ≥3 rats. Statistical analysis was performed by both ANOVA and unpaired t-test.

Rats by RT-PCR. On the basis of these observations, we propose that gastrin inhibits secretin-induced choleresis by interaction with specific receptors on cholangiocytes by decreasing SR gene expression and secretin-induced cAMP levels, two important regulatory determinants of ductal secretory activity (5, 7, 8, 27, 31, 53).

The intrahepatic biliary tree is the principal site for hormone-regulated ductal bile secretion in normal and pathophysiological states including BDL (1, 3–5, 7–10, 14, 15, 19, 27, 53). The gastrointestinal hormone secretin stimulates both in vivo (1, 3, 30, 31, 53) and in vitro (5, 8, 9, 27, 31, 53) ductal bile secretion by interacting with specific receptors located in rat liver solely on cholangiocytes (7). The interaction of secretin with its receptor leads to an increase in cAMP (5, 8, 30, 31, 53), the most important determinant of ductal secretory activity (5, 8, 27, 30, 31, 53). The increase in intracellular cAMP levels causes opening of Cl⁻/HCO₃⁻ exchanger activity (10, 19), which leads to the secretin-stimulated bicarbonate-rich choleresis (1, 3, 31, 53). In addition, VIP and bombesin stimulate ductal secretory activity both in vivo and in isolated intrahepatic bile duct fragments (14, 15). Also, Tietz et al. (51) have shown that somatostatin inhibits both secretin-induced cAMP and exocytosis in vitro and secretin-induced choleresis in vivo by interacting with specific (i.e., SSTR₂) receptors on cholangiocytes. In contrast to humans, where ductal bile secretion is 40% of total bile secretion (38), ductal bile flow in normal rats represents only 10% of total bile volume (3). SR gene expression is very low (7) and secretin does not increase bile secretion (1, 3, 20, 31, 53). In these studies, we employed the BDL model in which SR gene expression and intracellular cAMP levels are elevated in vitro (7, 8, 53) and in which secretin stimulates ductal bile secretion in vivo (1, 3, 53).

Gastrin, which stimulates gastric acid secretion and growth of the acid producing mucosa (21, 57), also has trophic effects in a number of organs including pancreas and intestine (18, 39, 45, 60). On the other hand, other investigators have shown that gastrin does not have stimulatory effects in the oxyntic mucosal D-cells (56) or in the liver (13). All these effects of gastrin on these organs occur by interaction with specific GRs (28, 36, 50, 59, 60). GRs have also been found in brain (58), colon cancer (44), and small cell cancer of the lung (47). Some studies (16, 17, 35) have shown that the liver is able to metabolize circulating gastrin (i.e., gastrin-17 and gastrin-14, but not gastrin-34). However, no data exist regarding the role of gastrin in the regulation of ductal secretory activity in rat liver. The present data indicate a direct interaction of gastrin on cholangiocytes (presumably through an interaction with specific receptors) for an explanation of the inhibitory effect of gastrin, both in vitro and in vivo, on secretin-stimulated ductal bile secretion. To acquire evidence for the presence of specific receptors for gastrin on cholangiocytes, we studied the dose-dependent effect of gastrin (from 10⁻⁷ to 10⁻¹⁰ M) on secretin-induced choleresis and bicarbonate output in vivo (Fig. 2, A and B) and secretin-induced cAMP synthesis in vitro (Fig. 7), which is a functional assay for the SR (5, 8, 20, 27, 31, 53). The presence of an inhibitory effect of gastrin on both secretin-induced choleresis and bicarbonate production (Fig. 6). Basal and hormone-induced intracellular adenosine 3',5'-cyclic monophosphate (cAMP) levels in pure preparations of cholangiocytes isolated from normal and BDL rat livers. After purification, cholangiocytes were incubated at 37°C for 1 h and then stimulated at 22°C for 5 min in the presence of 0.2% BSA with 1) gastrin (10⁻⁷ M); 2) gastrin plus secretin (both 10⁻⁷ M); 3) secretin (10⁻⁷ M); or 4) 0.2% BSA (control). *P < 0.05 vs. its corresponding value from normal rat liver. **Hormone-induced cAMP levels of cholangiocytes differ significantly from their corresponding basal levels (P < 0.05). Gastroin-modulated cAMP synthesis of cholangiocytes differs significantly from secretin-stimulated cAMP levels (P < 0.05). Data are means ± SE for ≥4 rats. Statistical analysis was performed by both ANOVA and unpaired t-test.

The intrahepatic biliary tree is the principal site for hormone-regulated ductal bile secretion in normal and pathophysiological states including BDL (1, 3–5, 7–10, 14, 15, 19, 27, 53). The gastrointestinal hormone secretin stimulates both in vivo (1, 3, 30, 31, 53) and in vitro (5, 8, 9, 27, 31, 53) ductal bile secretion by interacting with specific receptors located in rat liver solely on cholangiocytes (7). The interaction of secretin with its receptor leads to an increase in cAMP (5, 8, 30, 31, 53), the most important determinant of ductal secretory activity (5, 8, 27, 30, 31, 53). The increase in intracellular cAMP levels causes opening of Cl⁻/HCO₃⁻ exchanger activity (10, 19), which leads to the secretin-stimulated bicarbonate-rich choleresis (1, 3, 31, 53). In addition, VIP and bombesin stimulate ductal secretory activity both in vivo and in isolated intrahepatic bile duct fragments (14, 15). Also, Tietz et al. (51) have shown that somatostatin inhibits both secretin-induced cAMP and exocytosis in vitro and secretin-induced choleresis in vivo by interacting with specific (i.e., SSTR₂) receptors on cholangiocytes. In contrast to humans, where ductal bile secretion is 40% of total bile secretion (38), ductal bile flow in normal rats represents only 10% of total bile volume (3). SR gene expression is very low (7) and secretin does not increase bile secretion (1, 3, 20, 31, 53). In these studies, we employed the BDL model in which SR gene expression and intracellular cAMP levels are elevated in vitro (7, 8, 53) and in which secretin stimulates ductal bile secretion in vivo (1, 3, 53).

Gastrin, which stimulates gastric acid secretion and growth of the acid producing mucosa (21, 57), also has trophic effects in a number of organs including pancreas and intestine (18, 39, 45, 60). On the other hand, other investigators have shown that gastrin does not have stimulatory effects in the oxyntic mucosal D-cells (56) or in the liver (13). All these effects of gastrin on these organs occur by interaction with specific GRs (28, 36, 50, 59, 60). GRs have also been found in brain (58), colon cancer (44), and small cell cancer of the lung (47). Some studies (16, 17, 35) have shown that the liver is able to metabolize circulating gastrin (i.e., gastrin-17 and gastrin-14, but not gastrin-34). However, no data exist regarding the role of gastrin in the regulation of ductal secretory activity in rat liver. The present data indicate a direct interaction of gastrin on cholangiocytes (presumably through an interaction with specific receptors) for an explanation of the inhibitory effect of gastrin, both in vitro and in vivo, on secretin-stimulated ductal bile secretion. To acquire evidence for the presence of specific receptors for gastrin on cholangiocytes, we studied the dose-dependent effect of gastrin (from 10⁻⁷ to 10⁻¹⁰ M) on secretin-induced choleresis and bicarbonate output in vivo (Fig. 2, A and B) and secretin-induced cAMP synthesis in vitro (Fig. 7), which is a functional assay for the SR (5, 8, 20, 27, 31, 53). The presence of an inhibitory effect of gastrin on both secretin-induced choleresis and bicarbonate output (Fig.

Fig. 6. Basal and hormone-induced intracellular adenosine 3',5'-cyclic monophosphate (cAMP) levels in pure preparations of cholangiocytes isolated from normal and BDL rat livers. After purification, cholangiocytes were incubated at 37°C for 1 h and then stimulated at 22°C for 5 min in the presence of 0.2% BSA with 1) gastrin (10⁻⁷ M); 2) gastrin plus secretin (both 10⁻⁷ M); 3) secretin (10⁻⁷ M); or 4) 0.2% BSA (control). *P < 0.05 vs. its corresponding value from normal rat liver. **Hormone-induced cAMP levels of cholangiocytes differ significantly from their corresponding basal levels (P < 0.05). Gastroin-modulated cAMP synthesis of cholangiocytes differs significantly from secretin-stimulated cAMP levels (P < 0.05). Data are means ± SE for ≥3 rats. Statistical analysis was performed by both ANOVA and unpaired t-test.

Fig. 7. Dose-dependent effects of gastrin on secretin-induced cAMP levels in pure cholangiocytes from BDL rats. After purification, cholangiocytes were incubated at 37°C for 1 h and then stimulated at 22°C for 5 min in the presence of 0.2% BSA with 1) secretin (10⁻⁷ M); 2) gastrin (10⁻⁷-10⁻¹⁰ M) plus secretin (10⁻⁷ M); or 3) 0.2% BSA (control). *Gastrin-modulated cAMP synthesis of cholangiocytes differs significantly from secretin-stimulated cAMP levels (P < 0.05). **Secretin-stimulated cAMP levels of cholangiocytes from BDL rats differ significantly from basal cAMP levels (P < 0.05). Data are means ± SE for ≥4 rats. Statistical analysis was performed by both ANOVA and unpaired t-test.
2, A and B) and secretin-stimulated intracellular cAMP (Fig. 7) at a physiological dose [blood gastrin concentration of $10^{-9}$–$10^{-10}$ M in rats (29)] supports the concept of specific, physiologically relevant receptors for gastrin on cholangiocytes. Furthermore, we have also shown that in the presence of proglumide, a specific GR antagonist (26, 50, 59, 60), gastrin does not inhibit either secretin-induced cholestasis (Fig. 3A) or biliary bicarbonate output (Fig. 3B) in BDL rats, consistent with the concept that gastrin inhibition of secretin-induced ductal bile secretion occurs by interaction with specific receptors on cholangiocytes. Finally, further evidence for the presence of receptors on cholangiocytes comes from our in vitro molecular analysis (Fig. 4) showing that a 444-bp product, 100% homology to the published sequence for the GR gene (28), was detected in both normal and hyperplastic cholangiocytes. Upregulation of the genetic expression of GR in BDL rat liver may increase cholangiocyte sensitivity to gastrin, leading to inhibition of secretin-stimulated ductal bile secretion by gastrin in BDL rats. Our studies are the first report of the presence of GRs in the liver with evidence of an inhibitory effect of gastrin on GR gene expression and secretin-induced cAMP levels in vitro and secretin-stimulated bicarbonate-rich cholestasis in vivo.

Secretin and gastrin receptors are different with respect to organ or cell type distributions, binding of agonists or antagonists, second messenger systems, different physiological effects, and structures (57). On the basis of these differences, SR belongs to a receptor super family containing SR, VIP, and parathyroid hormone receptors (23, 24), whereas GR is in the family of CCK (CCK-A and CCK-B) receptors (11). GR employs Ca$^{2+}$-dependent pathways (55) and not cAMP as a second messenger system (33, 51). Consistent with its known signaling pathway (33, 51, 55), gastrin did not alter basal intracellular cAMP levels or basal bile flow in BDL rats (Figs. 1 and 6). The lack of effect of gastrin on both spontaneous bile secretion in vivo (Fig. 1) and basal cAMP levels in vitro (Fig. 6) contrasts with somatostatin, which inhibits both spontaneous ductal bile flow and basal cAMP levels in cholangiocytes from BDL rats (53).

We have considered a number of other potential effects of gastrin on ductal bile flow other than through interaction with GR on cholangiocytes. For example, gastrin may increase somatostatin levels or reduce endogenous secretin release and thereby inhibit ductal bile flow (51). Yet, in previous studies (46, 49) gastrin had no effect on the release of somatostatin or secretin from isolated cells or circulatory levels in vivo. We have considered that gastrin may interact with its closely related CCK-A receptor, but previous studies have shown that stimulation of CCK-A receptors with CCK leads to an increase in ductal bile secretion rather than the decrease (42) we observed in this study. Although it can be argued that proglumide, which we employed in these studies as a specific GR blocker, has some effects on CCK-A receptors (34), the more specific blocker L-$365,260$ could not be used because of its poor solubility and bioavailability in vivo (12, 35).

Our demonstration of secretin expression and physiological responses of GR in cholangiocytes has both physiological and pathophysiological relevance. In a fashion similar to that shown in the stomach (43), the opposing effects of secretin and gastrin also regulate ductal bile secretion. This study and others (1, 3–10, 14, 15, 19, 20, 27, 30, 31, 53) support the concept that the intrahepatic biliary epithelium is subjected to a very tight hormonal regulation, with stimulatory effects exerted by secretin (1, 3, 5, 8, 31, 53), bombesin (14), and VIP (15) and an opposing inhibition by somatostatin (53) and gastrin (20). Because preliminary data (20, 32) indicate that, similar to somatostatin (6, 52, 54), gastrin also exerts inhibitory effects on cholangiocyte proliferation, the regulation of secretory processes could be tightly coupled with that of proliferation and thus explain the reason for the involvement of many different hormones and/or neuropeptides. The inhibitory effects of gastrin on both secretin-induced cAMP synthesis and biliary bicarbonate excretion suggest that gastrin may affect (in addition to secretin and its receptor) the function of a number of hormone-regulated ion transporters (e.g., cystic fibrosis transmembrane regulator and Cl$^{-}$/HCO$_3$$^{-}$ exchanger). Finally, the presence of GR on cholangiocytes opens up new therapeutic windows for the diagnosis and treatment of cholangiocarcinoma. GR could be used similarly to somatostatin receptor (52) to image cholangiocarcinomas with radiolabeled ligands.

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