Regulation of cholangiocyte bicarbonate secretion

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Regulation of cholangiocyte bicarbonate secretion. Am J Physiol Gastrointest Liver Physiol 281: G612–G625, 2001.—The objective of this review article is to discuss the role of secretin and its receptor in the regulation of the secretory activity of intrahepatic bile duct epithelial cells (i.e., cholangiocytes). After a brief overview of cholangiocyte functions, we provide an historical background for the role of secretin and its receptor in the regulation of ductal secretion. We review the newly developed experimental in vivo and in vitro tools, which lead to understanding of the mechanisms of secretin regulation of cholangiocyte functions. After a description of the intracellular mechanisms by which secretin stimulates ductal secretion, we discuss the heterogeneous responses of different-sized intrahepatic bile ducts to gastrointestinal hormones. Furthermore, we outline the role of a number of cooperative factors (e.g., nerves, alkaline phosphatase, gastrointestinal hormones, neuropeptides, and bile acids) in the regulation of secretin-stimulated ductal secretion. Finally, we discuss other factors that may also play an important role in the regulation of secretin-stimulated ductal secretion.

IN THE LIVER TWO TYPES of epithelia (i.e., hepatocytes and cholangiocytes) contribute to bile secretion (12, 27, 34, 73, 105, 132, 135, 136, 140). Separate hepatic (105) and ductular transport mechanisms (6, 8, 10, 12, 13, 16, 18, 20, 27, 34, 43–46, 63, 75) allow for regulation of bile volume and composition required for changing physiological needs. The bile acid-dependent flow derived from hepatocyte canalicular secretion accounts for 30–60% of spontaneous basal bile flow (105). A canalicular bile acid-independent secretion, probably caused by transport into bile of organic solutes (glutathione) and inorganic electrolytes, accounts for 30–60% of basal bile flow (105). At the level of the bile ducts, both secretion and reabsorption of fluid and inorganic electrolytes modify canalicular bile (6, 10–13, 16, 22, 27, 34, 43–46, 63, 75, 93–95, 119, 120, 135, 143). Ductal secretion chiefly occurs in response to secretin and represents 30% of basal bile flow in humans and 10% in rats (11, 140). Glutathione is present in bile but is almost quantitatively broken down within the biliary tree by hepatic γ-glutamyltransferase (1, 90, 112).

Cholangiocytes possess specific membrane transport systems for a large number of substrates. For example, cholangiocytes absorb glucose (81), bile acids (8, 51, 80), and amino acids (50, 125) from bile. Human cholangiocytes are also involved in the transport of secretory component and IgA into bile (137).

The secretory/absorptive properties of the intrahepatic biliary epithelium are supported by the presence of microvilli on their apical pole (31, 71, 79). Ductal bile secretion is coordinately regulated by a number of factors including nerves (16, 83), enzymes [e.g., alkaline phosphatase (AP) (17)], gastrointestinal hormones [e.g., secretin (10, 143), somatostatin (143), and gastrin (63)], peptides [endothelin-1 (ET-1) (38), bombesin, and vasoactive intestinal polypeptide (VIP) (43, 46)], and bile salts (5, 7) (Table 1).
Pathologically, cholangiocytes are the target cells in a number of chronic cholangiopathies including primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), diseases that are associated with cholangiocyte proliferation and/or loss (12, 120). In rodents, cholangiocyte proliferation/loss is achieved by a number of pathological maneuvers including bile duct ligation (BDL) (10, 12, 62, 63, 83, 85, 143), experimental cirrhosis [induced by chronic administration of CCl₄ (4) or phenobarbital in conjunction with CCl₄ (76)], experimental fascioliasis [induced by oral administration of 20 metacercariae of Fascioliasis hepatica (88)], partial hepatectomy (84), acute CCl₄ administration (85, 86), vagotomy (83), and chronic feeding of bile acids (7) or the toxin α-naphthylisothiocyanate (ANIT) (11, 32) (Table 2). These models of bile duct hyperplasia/ductopenia are closely associated with increases (e.g., after BDL, ANIT feeding, oral administration of F. hepatica, cirrhosis, or partial hepatectomy; Refs. 7, 9–11, 32, 62, 63, 83, 84, 88, 143) or decreases (e.g., after CCl₄ administration or vagotomy; Refs. 83, 85, 86) in ductal secretion (Table 2).

### Table 1. Factors regulating secretin-stimulated ductal secretion

<table>
<thead>
<tr>
<th>Regulatory Factors</th>
<th>Effect on Secretin-Stimulated Ductal Secretion</th>
<th>Second Messenger</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholinergic nerves</td>
<td>Stimulation of M₃ cholinergic receptors increases secretin-stimulated ductal bicarbonate secretion</td>
<td>Secretin-stimulated cAMP levels ↑</td>
<td>16</td>
</tr>
<tr>
<td>Interruption of cholinergic innervation (by vagotomy)</td>
<td>Inhibition of SR gene expression and secretin-stimulated bicarbonate-rich choleretosis</td>
<td>Secretin-stimulated cAMP levels ↓</td>
<td>83</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Inhibition of secretin-stimulated bile flow and bicarbonate secretion</td>
<td>Secretin-stimulated cAMP levels ↓</td>
<td>17</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>Inhibition of SR gene expression, secretin-stimulated bile flow, bicarbonate secretion, and exocytosis by interaction with SSTR₂ receptors</td>
<td>Secretin-stimulated cAMP levels ↓</td>
<td>9, 72, 117, 118, 143</td>
</tr>
<tr>
<td>Gastrin</td>
<td>Inhibition of SR gene expression and secretin-stimulated bile and bicarbonate secretion by interaction with CCR-B/gastrin receptors</td>
<td>IP₃, PKC ↑ Membrane translocation of PKC-α PKC-dependent inhibition of secretin-stimulated cAMP levels</td>
<td>62, 63</td>
</tr>
<tr>
<td>ET-1</td>
<td>Inhibition of SR secretin-stimulated bile and bicarbonate secretion by interaction with ET₃ receptors</td>
<td>Secretin-stimulated cAMP levels ↓</td>
<td>38, 56</td>
</tr>
<tr>
<td>VIP</td>
<td>In humans, increases secretin choleresis</td>
<td>Not determined</td>
<td>110, 111</td>
</tr>
<tr>
<td></td>
<td>In dogs, sheep, and rats, does not alter secretin choleresis</td>
<td>cAMP independent</td>
<td>43, 65, 92</td>
</tr>
<tr>
<td>Bombesin</td>
<td>In dogs, induces choleresis through increased secretin release</td>
<td>Not determined</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>In rats, does not alter secretin choleresis</td>
<td>cAMP independent</td>
<td>44, 46</td>
</tr>
<tr>
<td>Substance P</td>
<td>In dogs, inhibits choleresis</td>
<td>Not determined</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>In rats, does not alter secretin choleresis</td>
<td>cAMP independent</td>
<td>21</td>
</tr>
<tr>
<td>Bile acids</td>
<td>TC and TLC increase secretin-stimulated ductal bicarbonate secretion</td>
<td>Secretin-stimulated cAMP levels ↑</td>
<td>5, 7</td>
</tr>
<tr>
<td>IL-5</td>
<td>Inhibits Cl⁻ channel currents by interaction with IL-5 receptors</td>
<td>Not determined</td>
<td>99</td>
</tr>
</tbody>
</table>

Most of the findings summarized here come from studies performed in rats, mice, sheep, dogs, and humans. SR, secretin receptor; IP₃, D-myo-inositol 1,4,5-trisphosphate; PKC, protein kinase C; ET-1, endothelin-1; TC, taurocholate; TLC, tauroliothocholate; IL-5, interleukin-5.

### MORPHOLOGY OF INTRAHEPATIC BILIARY EPITHELIUM

Reviews on cholangiocyte secretory functions are available (12, 19, 27, 34, 73, 120, 132, 135, 136, 140); however, none has specifically focused on the role and mechanisms of action of secretin in the regulation of cholangiocyte secretion. The biliary duct system has been classified into three segments based on duct size (12, 89, 124). This classification includes extrahepatic bile ducts, large bile ducts, and intrahepatic small bile ducts or ductules (12, 89, 124). At the functional level, hepatocyte bile is transferred from the bile canaliculus to the smallest bile ducts (<5 μm in external diameter) through the duct of Hering (12, 89, 124). Small intrahepatic bile ducts (lined by 4–5 cholangiocytes) are characterized by the presence of a basement membrane, tight junctions between cells, and microvilli projecting into the duct lumen (12, 89, 124). Like small branches of a tree, small bile ducts join into intralobular ducts ranging from 20 to 100 μm in cross-sectional diameter (12, 89, 124). In larger bile ducts the lining...
cholangiocytes are progressively larger and more columnar in shape (12, 89, 124).

**SECRETIN FUNCTIONS IN THE BODY**

Secretin [a 27-amino acid neuroendocrine peptide synthesized by specific endocrine cells, S cells, localized mainly in the mucosa of the duodenum and proximal jejunum (42, 152, 153)] regulates the physiological functions of many organs including brain [e.g., activation of tyrosine hydroxylase activity (69)], pancreas, intestine, and liver (9–11, 39, 85, 86, 153). Secretin stimulates the gastric secretion of pepsin and inhibits the secretion of gastric acid and food-stimulated gastrin from G cells in the gastric antrum (152, 153). Furthermore, secretin affects the motility of the small intestine, decreases lower esophageal sphincter pressure, relaxes the sphincter of Oddi, and inhibits postprandial emptying (152, 153). Secretin increases heart rate and causes dilatation of peripheral blood vessels (29, 104).

**HISTORICAL BACKGROUND**

Secretin was originally discovered by Bayliss and Starling, who demonstrated that this hormone stimulates pancreatic secretion and bile flow in dogs (28). In vivo studies in dogs with BDL by Rous and McMaster (122) showed that cholangiocytes secrete water and electrolytes in ductal bile. Andrews (23) proposed a secretory model in which hepatocytes were responsible for the hepatic metabolic activity, whereas the intrahepatic biliary tree represented the main secretory unit of the liver. On the basis of measurement of electrolyte excretion in dog bile, Wheeler et al. (156) identified two distinct anatomic secretory sites, one responsible for bile acid-dependent bile flow and one (more distal) important for the choleretic effect of secretin. In support of the secretory capacity of the ductal epithelium, studies in dogs and rabbits have shown secretion of water and electrolytes by an isolated segment of the extrahepatic bile duct in situ (103, 131) and in vitro (40). Studies in dogs with bile fistula (155) and isolated, perfused pig liver (66) have shown that secretin increases bicarbonate-rich bile secretion and that the increase in bile flow is proportional to the logarithm of the dose of secretin. Furthermore, studies in isolated, perfused pig liver demonstrated that the electrolyte composition of the fraction of bile stimulated by secretin is independent of the bile flow before secretin administration in the perfused preparation (67). Other studies in dogs have shown that 1) infusion of secretin via the hepatic artery [the major blood supply of the intrahepatic biliary epithelium (37, 115)] induces greater choleresis compared with secretin infusion through the splenic vein and 2) the biliary tree volume was smaller during secretin choleresis compared with taurocholate-induced choleresis (154). These studies suggest that secretin-stimulated bicarbonate-rich bile flow derives from the interaction of this hormone with intrahepatic bile ducts rather than hepatocytes. However, other studies have shown that interruption of blood flow to the intrahepatic biliary epithelium (by short-term ligation of the hepatic artery of guinea pigs) does not alter cholangiocyte secretory activity (142). Radiolabeled mannitol and erythritol (used with the assumption that these carbohydrates are transported across the bile canaliculus but not the biliary epithelium) have been used to define the anatomic site of secretin-induced choleresis in guinea pigs (55). However, recent studies in rats (128) and guinea pigs (141) have questioned the use of these two molecules (to distinguish between hepatocyte and cholangiocyte bile secretion), because they can cross the intrahepatic biliary epithelium. For example, erythritol is not an ideal marker of canicular bile flow because it has been shown to cross the rat intrahepatic ductal epithelium, possibly via intercellular junctions (128). In vivo studies in humans, baboons, and dogs have also shown that intravenous infusion of secretin increases bile flow (87). These studies also showed that secretin-induced choleresis is associated with an increase in cAMP levels in extrahepatic bile duct tissue in humans and baboons (but not dogs), suggesting that cAMP may be a second messenger system for secretin (87). Conclusive evidence for the role of secretin in directly stimulating

### Table 2. Relationship between bile duct hyperplasia/ductopenia and secretin-regulated ductal secretion

<table>
<thead>
<tr>
<th>Models</th>
<th>Proliferation</th>
<th>Loss</th>
<th>Secretin-Induced Ductal Secretion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDL</td>
<td>↑</td>
<td>↔</td>
<td>↑</td>
<td>9–12, 15, 62, 63, 83, 85, 143</td>
</tr>
<tr>
<td>Partial hepatectomy</td>
<td>↑</td>
<td>↔</td>
<td>↑</td>
<td>84</td>
</tr>
<tr>
<td>Acute CCl₄ administration</td>
<td>↑</td>
<td>↔</td>
<td>↑†</td>
<td>85, 86</td>
</tr>
<tr>
<td>Oral administration of Fasciolasis hepatica</td>
<td>↑</td>
<td>Not determined</td>
<td>↑</td>
<td>88</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>↑</td>
<td>Not determined</td>
<td>↑</td>
<td>4, 76</td>
</tr>
<tr>
<td>ANIT feeding</td>
<td>↑</td>
<td>↔</td>
<td>↑</td>
<td>11, 32</td>
</tr>
<tr>
<td>Bile acid feeding (TC and TLC)</td>
<td>↑</td>
<td>↔</td>
<td>↑</td>
<td>7</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>↓</td>
<td>↑</td>
<td></td>
<td>83</td>
</tr>
</tbody>
</table>

The data presented in this table were obtained in rat and mouse models of ductal hyperplasia. BDL, bile duct ligation; ANIT, α-naphthylisothiocyanate. *Delayed compensation for loss of ducts; †Caused by cholangiocyte apoptosis.
ductal secretion came from recent studies (2, 3, 10, 11, 76) showing that in vivo secretin induces a massive bicarbonate-rich choleresis [usually absent under normal conditions (10, 11, 63, 84, 143)] in rats with enhanced intrahepatic ductal mass induced by BDL (2, 3, 10, 11), cirrhosis (76), and chronic α-naphthylisothiocyanate (ANIT) feeding (11, 32). Secretin-stimulated choleresis results from the direct interaction of secretin with secretin receptors [SR; exclusively expressed by cholangiocytes in rat liver (15, 53)]. The interaction of secretin with its receptors induces an increase in intracellular cAMP levels (6, 9, 13, 14, 62, 63, 75, 83–86, 143) and activation of the Cl− channel cystic fibrosis transmembrane regulator (CFTR), with subsequent activation of the Cl−/HCO3− exchanger leading to bicarbonate secretion in ductal bile (Fig. 1).

**EXPERIMENTAL TOOLS FOR EVALUATING SECRETIN-STIMULATED DUCTAL SECRETION**

**In Vivo Models of Cholangiocyte Hyperplasia/Ductopenia**

Recently, a number of animal models of ductal hyperplasia [inducing an increase in the number of intrahepatic bile ducts (7, 10, 11, 62, 83, 84) or loss of cholangiocytes (85, 86)] have been developed and have allowed us to increase our knowledge of the role and mechanisms of action of secretin in the regulation of ductal secretion (Refs. 7, 10–13, 15, 62, 63, 76, 83–86, 88, 143; see Table 2). Rodent models of cholangiocyte hyperplasia include partial hepatectomy (84), chronic feeding of bile acids [i.e., taurocholate (TC) and taurolithocholate (TLC); Ref. 7] or ANIT (11, 32), cirrhosis [induced by chronic administration of phenobarbital in conjunction with CCl4 to rats (76) or chronic CCl4 treatment in mice (4)], experimental fascioliasis (88), and BDL (9, 10) (Table 2). These models of duct hyperplasia are closely associated with increased secretin-stimulated ductal secretion evidenced by 1) increased SR gene expression (7, 9, 14, 15, 63, 84), secretin-stimulated cAMP levels (7, 9, 14, 62, 63, 84, 143), and Cl−/HCO3− exchanger activity (16, 84) in purified cholangiocytes and 2) enhanced secretin-stimulated bicarbonate-rich choleresis in vivo (10, 11, 63, 76, 83, 84, 88, 143).

Animal models of ductopenia [i.e., total vagotomy (83)] or liver toxins [i.e., CCl4 (85, 86)] result in a decrease or loss of secretin-stimulated ductal secretion. Interruption of the cholinergic innervation (by total vagotomy of BDL rats) inhibits SR gene expression, secretin-stimulated cAMP levels, and secretin-induced bile flow and bicarbonate secretion (83). Maintenance of cAMP levels by chronic forskolin administration to BDL rats prevents the inhibitory effects of vagotomy on secretin-stimulated cholangiocyte secretion (83). In rats, the toxin CCl4 has been shown to selectively damage large cholangiocytes (with loss of secretin-stimulated secretory responses), whereas small cholangiocytes are resistant to CCl4-induced duct damage and, de novo, express SR and respond physiologically to secretin to compensate for the loss of secretin-induced secretion of large ducts (85, 86).

**In Vitro Tools**

A major advancement in the understanding of the role and mechanisms of action of secretin in the regulation of ductal secretion came from the development of sophisticated techniques [e.g., immunoaffinity separation (71, 75) and micropipetting (6, 100, 119)] for the isolation and phenotypic characterization of pure preparations of pooled small and large cholangiocytes and intrahepatic bile duct units (IBDU) from normal and cholestatic rat liver (6, 10, 11, 13, 45, 62, 63, 71, 75, 83, 84, 143). Isolated cholangiocytes and IBDU have allowed us to evaluate the effect of secretin on cAMP levels (6, 9, 13, 14, 16, 62, 63, 75, 83–86, 98, 143), protein kinase A (PKA) activity (16, 98), Cl− channel
conductance (14, 54, 98), Cl⁻/HCO₃⁻ exchanger activity (13, 16, 18, 84, 134), and water channel activity (93, 95). IBDU have the advantage (over isolated cholangiocytes) of maintaining cell polarity, permitting direct assessment of secretin-stimulated ductal secretory activity by changes in ductal lumen and the maintenance of polarized epithelial structure (6, 38, 100, 119). Other advantages of the IBDU model include the ability to microinject molecules directly into the duct lumen or perfusion of the duct lumen (6, 97, 100, 119). Primary cultures of rat cholangiocytes (151, 157) (which retain phenotypic and functional characteristics of biliary origin) are pathophysiologically important tools and have allowed us to define the role of cAMP after stimulation with forskolin (151), a CAMP activator (77, 83). Studies by McGill et al. (98) used a sophisticated approach (i.e., patch-clamp recording technique in whole cells) to demonstrate cAMP-dependent secretin stimulation of membrane Cl⁻ channel (i.e., CFTR) activity in isolated rat cholangiocytes. Another important tool for evaluating the mechanisms of secretin-stimulated ductal secretion is represented by the isolation (by isopycnic centrifugation on sucrose gradients) and characterization of apical and basolateral plasma membrane vesicles from polarized normal rat cholangiocyte cultures (144) and freshly isolated cholangiocytes isolated from BDL rats (61, 145). The development of this tool has allowed us to obtain important mechanistic information on the role of secretin on ductal water channel activity (93–95).

SECRETIN RECEPTOR

Secretin receptors belong to a unique family of G protein-coupled receptors including receptors for VIP, pituitary adenylate cyclase-activating polypeptide, gastric inhibitory peptide, glucagon, glucagon-like peptide 1, calcitonin, calcitonin gene-related peptide, parathyroid hormone, growth hormone-releasing factor, and corticotropin-releasing factor (114). The SR (prototypic of the class II family of G protein-coupled receptors) contains a long extracellular amino-terminal domain containing six highly conserved Cys residues and one Cys residue (Cys(11)) (25). Recent studies have identified the specific structural and functional domain of the secretin and its receptor (113). These studies have shown that the amino-terminal 15 residues of secretin are critical for the stimulation of SR (113). The studies have also shown that the amino terminus of the SR is necessary, but not sufficient, requiring the complementation of an extracellular loop domain for the physiological response to secretin (113). Secretin binding to the extracellular domain of SR results in coupling with heterotrimeric G proteins at a cytosolic domain of SR (58).

The G proteins consist of α-, β-, and γ-subunits (114). G proteins are members of a superfamily of GTPases that are fundamentally conserved from bacteria to mammals and play a role in many aspects of cell regulation (114). Ligand-bound SR activates G proteins, which in turn activate adenyl cyclase, leading to increased intracellular cAMP accumulation (114). Characterization of hormone-binding domains of SR and SR cloning and expression is beyond the scope of this review and has been recently discussed in detail elsewhere (148).

Recently, the cDNA for the human (47), rat (70), and rabbit (138) SR gene has been cloned and functionally characterized. SR is widely distributed in the body including brain, heart, stomach, intestine, and pancreas (33, 57, 60, 70). In the liver, in situ autoradiographic studies of liver sections (53) and in vitro studies in purified cholangiocytes (15) and cholangiocyte membranes (52) have shown that SR are exclusively expressed on the basolateral membrane of rat cholangiocytes.

MECHANISMS OF SECRETIN-REGULATED DUCTAL SECRETION

Secretin stimulates ductal secretion by a series of sequential and coordinated events (6, 10–16, 18, 22, 54, 63, 75, 84, 134, 143). First, secretin interacts with basolateral SR (95), expressed only by cholangiocytes in rat liver (15, 53). This interaction induces elevation of cytosolic cAMP levels, activation of intracellular PKA (22, 41), and opening of the cAMP-dependent Cl⁻ channels by phosphorylation (41), leading to extrusion of Cl⁻ ions with subsequent depolarization of the cell membrane (54, 98) (Fig. 1). Opening of Cl⁻ channels (14, 54, 98) induces a Cl⁻ gradient favoring the activation of the apically located Cl⁻/HCO₃⁻ exchanger (13, 18, 84, 129, 134), which results in secretin-stimulated bicarbonate-rich cholestasis (10, 11, 63, 84, 143) (Fig. 1). The electronegative Na⁺–HCO₃⁻ symport is activated at the basolateral domain of a rat cholangiocyte cell line (129), as a consequence of depolarization induced by Cl⁻ efflux, and this causes the entry of HCO₃⁻ into cells. Studies in purified rat cholangiocytes have shown (18) that the Na⁺–HCO₃⁻ symport counters bicarbonate excretion by working as an acid extruder, thus contributing to maintenance of intracellular pH. Secretin directly stimulates the opening of the CFTR channel but does not directly regulate either the Na⁺–HCO₃⁻ or the Na⁺/H⁺ exchanger (NHE) of rat cholangiocytes (18). The NHE3 isoform (which has been recently localized...
to the apical membrane of mouse and rat cholangiocytes) plays an important role in fluid secretion and absorption from bile duct lumen (101). In pigs, cholangiocyte bicarbonate secretion is also dependent on carbonic anhydrase II, an enzyme that catalyzes hydration of carbon dioxide to bicarbonate and hydrogen ions (36). Insertion of CFTR or Cl-/HCO₃⁻ exchanger into the apical membrane domain of cholangiocytes from intracellular cytoplasmic stores enhances their activity (20). Other studies in guinea pigs have shown that secretin-induced cholestasis is caused by the activation of NHE by secretin, whose activation causes intracellular alkalinization in cholangiocytes (68). Recent studies in cultured human intrahepatic cholangiocytes have shown that human cholangiocytes express two acid exuders (i.e., Na⁺/H⁺ exchanger and Na⁺-dependent Cl⁻/HCO₃⁻ exchanger) and an acid loader (i.e., Cl⁻/HCO₃⁻ exchanger) but not the cAMP-dependent H⁺-ATPase (133). These studies show that HCO₃⁻ influx is regulated by Cl⁻/HCO₃⁻ exchange, whereas Na⁺-HCO₃ cotransport is inactive at physiological pH (133). The studies have also shown that stimulation of Na⁺-independent Cl⁻/HCO₃⁻ exchanger by cAMP does not require activation of Cl⁻ conductance. Other studies in pigs (64, 150) have proposed the role of H⁺-ATPase in the regulation of ductal secretion, because H⁺-ATPase is inserted into the basolateral cholangiocyte membrane in response to secretin, thus working as an acid exuder. However, recent studies in rats have shown that intrahepatic IBDU do not express H⁺-ATPase (44).

Recent studies have shown that the PKA system regulates secretin-stimulated bicarbonate-enriched ductal secretion (22). These studies have shown that Sp-adenosine 3',5'-cyclic monophosphothiolate (Sp-cAMPS), a PKA-specific agonist, stimulates ductal bicarbonate secretion, whereas Rp-cAMPS, a specific PKA inhibitor, decreases secretin-stimulated ductal bicarbonate secretion (22). The data suggest that secretin-induced cholestasis is regulated, at the level of CFTR, by a balance between the activities of kinases [inducing activation (22)] and phosphatases [causing inactivation (17)].

Other studies in rat cholangiocytes have shown that secretin stimulates insertion of transporters into the apical membrane of cholangiocytes via a CAMP-dependent but cGMP-, d-myo-inositol 1,4,5-trisphosphate (IP₃)-, and Ca²⁺-independent mechanism (75, 143).

The transport of water to ductal bile is regulated by membrane aquaporin water channels (AQP) present in both the basolateral and apical domains of rat (93, 95, 108, 121) and human (107) intrahepatic cholangiocytes. In a fashion similar to the urinary system, in which aquaporin activity in the collecting tubule and bladder epithelium is modulated by vasopressin (149), membrane water channels are also regulated in the rat intrahepatic biliary epithelium (93, 95). In rats, secretin increases water channel activity in the cholangiocyte apical membrane by stimulating the movement of latent inactive AQP1 (associated with internal cholangiocyte cytoplasmic vesicles) to the cholangiocyte apical membrane, where they become active water channels (93, 95). Secretin-stimulated AQP1 insertion into rat cholangiocyte apical membrane is microtubule-dependent because it is inhibited by pretreatment of cholangiocytes with colchicine (but not with its inactive analog β-lumicolchicine). These studies demonstrated that secretin-induced secretion of water and electrolytes is dependent on activation of latent AQP1 in rat cholangiocytes (93, 95). Recent studies have also localized AQP4 in the basolateral membrane of rat cholangiocytes (94). In contrast to AQP1, which is targeted to the apical cholangiocyte membrane by secretin (93, 95), AQP4 is not regulated by secretin (94). AQP4 may be important in facilitating basolateral transport of water in cholangiocytes, an important step in the regulation of bile secretion. Recent studies in Xenopus oocytes injected with CFTR demonstrated the presence of CFTR in vesicles and cAMP-dependent (by forskolin treatment) membrane insertion of these CFTR-containing vesicles (139). The apical membrane insertion of CFTR from these vesicles may provide a link between activation of CFTR and cAMP-dependent (by secretin activation) regulation of ductal secretion of water and electrolytes.

Factors Regulating Secretin-Stimulated Ductal Secretion

Parasympathetic Innervation

In the liver, sympathetic and parasympathetic innervation originate from the celiac ganglion (sympathetic) and from the vagus nerve (parasympathetic) (116, 147) and innervate the hepatic artery, the portal vein, the intrahepatic and extrahepatic biliary epithelium, and parenchymal cells (116, 147). Recent studies in rats have shown that the parasympathetic system regulates secretin-stimulated ductal secretion (16, 106). Nathanson et al. (106) demonstrated that ACh elicits both Ca²⁺ increase and oscillation in rat IBDU and isolated cholangiocytes because of both influx of extracellular Ca²⁺ and mobilization of thapsigargin-sensitive Ca²⁺ stores. Other studies have shown that intrahepatic parasympathetic terminations release ACh, which interacts selectively with M₃ ACh receptors on cholangiocytes, inducing an increase in secretin-stimulated cholangiocyte cAMP synthesis and Cl⁻/HCO₃⁻ exchanger activity by Ca²⁺-calcinemium-mediated, PKC-independent modulation of adenyl cyclase (16). In support of the concept that cholinergic nerves regulate secretin-stimulated ductal secretion, interruption of the parasympathetic innervation (by total vagotomy) in BDL rats decreases SR gene expression and secretin-stimulated bile flow and bicarbonate secretion through a decrease in M₃ ACh receptor expression (83).

Alkaline Phosphatase

AP is a nonspecific protein phosphatase whose precise function is unknown. Elevated serum AP levels are observed in cholestatic liver diseases (10, 123). Cholangiocytes are continuously exposed at their apical mem-
brane to high concentrations of AP in bile (74, 78). Recently, the effects of acute and chronic administration of AP on secretin-stimulated ductal secretion were evaluated in vivo in rats with bile fistula and in vitro in purified rat IBDU (17). In vivo, acute and chronic administration of AP decreased both basal and secretin-stimulated bile flow and biliary bicarbonate secretion in BDL rats (17). In vitro, basal and secretin-stimulated Cl-/HCO₃⁻ exchanger activity of rat IBDU was immediately inhibited by AP intraluminal microinjection (apical exposure) but only after a prolonged exposure to the basolateral domain of cholangiocytes (17). The inhibitory effect of AP (which is transcytosed from serum to cholangiocyte apical membrane) on secretin-stimulated ductal secretion may be due to its capacity to block CFTR activity or to hydrolyze ATP bonds (17). The findings suggest that elevated serum and biliary AP levels may be not only the result of cholestasis but also an adaptive reaction for decreasing secretory activity during bile duct obstruction (17).

Gastrointestinal Hormones

Gastrin. Gastrin modulates the functions of several epithelia by interaction with CCK-B/gastrin receptors through Ca²⁺-, IP₃-, and protein kinase C (PKC)-dependent mechanisms (see Fig. 2) (159, 160). In the liver, gastrin inhibits secretin-stimulated ductal secretion of BDL rats at the physiological doses of 10⁻⁹ – 10⁻⁷ M (63). The presence of an inhibitory effect of gastrin on secretin-induced ductal secretion at a physiological dose [blood gastrin concentration of 10⁻⁹ – 10⁻¹⁰ M in rats (82)] supports the presence of specific, physiologically relevant receptors for gastrin on cholangiocytes. We suggest that gastrin [similar to somatostatin (9, 143)] may be physiologically important in the regulation of enhanced secretin-stimulated ductal secretion in cholestatic liver diseases by counterpoising the stimulatory effects of secretin. The inhibitory effect of gastrin on secretin-stimulated ductal secretion occurs through activation and membrane translocation of the Ca²⁺-dependent PKC-α (62). These data suggest that cross-talk between the cAMP/PKA system [by which secretin stimulates ductal secretion (9, 16, 62, 63, 84–86)] and the IP₃/PKC system [by which gastrin inhibits secretion and proliferation (62, 159, 160)] may play an important role in the regulation of overall cholangiocyte secretory activity.

Somatostatin. Studies in dogs (117) and in rats (118, 143) showed that somatostatin inhibits both basal and secretin-stimulated bicarbonate-rich cholestasis by inhibition of exocytic vesicle insertion into cholangiocyte apical membranes (Refs. 75 and 143; Fig. 2) through interactions with a subtype (i.e., SSTR₂) of somatostatin receptors (143). These studies also showed that secretin-stimulated insertion of transporters into the apical membrane of rat cholangiocytes is dependent on the microtubule system because it is inhibited by pretreatment of cholangiocytes with colchicine (75, 143). In rats, somatostatin inhibition of secretin-stimulated ductal secretion is also associated with decreased SR gene expression (9) and decreased secretin-stimulated cAMP levels (9, 143). The inhibitory effect of somatostatin on secretin-stimulated ductal secretion is more evident in animal models of ductal hyperplasia (e.g., BDL), in which there is upregulation of SR and enhanced secretin-stimulated cAMP levels. On the basis of these findings (75, 143), the “membrane microdomain recycling model” (Fig. 3) has been proposed in rat liver to explain the cooperative interactions between secretin and somatostatin in the regulation of ductal secretory activity. In contrast to these findings, other studies in rats showed that colchicine does not inhibit secretin-induced ductal secretion, thus providing evidence against a pivotal role of exocytic vesicle insertion into cholangiocyte apical membrane to explain the choleric effect of secretin (49).

Peptides

Endothelin. ET-1, a polypeptide containing 21 amino acids, has multifunctional properties in several organs...
Recent studies in rats showed that ET-1 receptors ETA and ETB are expressed by cholangiocytes and that ET-1 inhibits SR gene expression, secretin-stimulated ductal lumen expansion, and secretin-induced cAMP levels by selective interaction with ETA but not ETB receptors (38). Furthermore, studies in primary cultures of human gallbladder epithelial cells showed that ET-1, via a Gi protein-coupled receptor, inhibits secretin-stimulated cAMP-dependent electrolyte secretion (56).

**Vasoactive intestinal peptide.** In vivo studies in humans showed that VIP potentiates the choleretic effect of secretin on bile flow and bicarbonate secretion (110, 111). Variation among species may explain the different cooperative interactions between VIP and secretin in the regulation of ductal bile secretion (43, 65, 92). In dogs, for example, VIP stimulated basal biliary secretion but did not alter the maximal effect of secretin on ductal secretion (92). In sheep liver, whereas secretin stimulated bile flow, VIP had no effect on ductal secretion (65). Although VIP regulates secretory activity of other epithelia through the cAMP/PKA (35, 102) or IP3/PKC pathway (130), recent studies in rats showed that VIP stimulates basal (but not secretin-stimulated) fluid and bicarbonate secretion via cAMP-independent pathways in IBDU (43). Together, the data suggest that VIP regulates basal and (possibly secretin stimulated) (110, 111) ductal secretion through a signaling pathway different from that of secretin.

**Bombesin.** In support of a possible interaction between bombesin and secretin, Kaminski and Deshpande (72) showed in dogs that bombesin markedly increases the bicarbonate-rich choleretic effect produced by intraduodenal acid infusion through increased secretin release. Recent studies in rats showed that bombesin increases ductal secretion and that bombesin stimulation of Cl⁻/HCO₃⁻ exchanger activity was independent of the increase in the second messengers cAMP, cGMP, and cytosolic Ca²⁺ (44, 46). Bombesin-stimulated biliary secretion is dependent on anion exchangers, Cl⁻ and K⁺ channels, and carbonic anhydrase but not microtubules (46), through mechanisms different from those established for secretin (6, 9–18, 22, 38, 62, 63, 83–87, 98, 143).

**Substance P.** Studies in rats showed that substance P decreases basal and secretin-stimulated secretion of pancreatic ducts (24). Similarly, in vivo studies in dogs showed that substance P inhibits secretin-stimulated choleretic effect (91). In contrast to these observations, preliminary studies in rat IBDU showed that substance P does not alter the effect of secretin on water and electrolyte secretion (21).

**Bile Acids**

A number of studies in rats showed that certain bile acids enter cholangiocytes through the Na⁺-dependent apical bile acid transporter (ABAT) (8, 80), thus modifying secretin-stimulated ductal bile secretion (5, 7, 8). For example, recent studies showed that TC and TLC increased in vitro (5) and in vivo (after chronic feeding) (7) secretin SR gene expression, secretin-stimulated cAMP levels, and secretin-stimulated bicarbonate-rich choleretic of normal rats.

**Nitric Oxide**

Recent studies by Trauner et al. (146) demonstrated that nitric oxide and cGMP, which stimulate secretion of rat hepatocyte couplets, do not alter basal or secretin-stimulated ductal lumen volume and Cl⁻/HCO₃⁻ exchanger activity of IBDU.

**Cytokines**

A number of studies indicate that the intrahepatic cholangiocytes and peribiliary gland in normal human livers and in hepatolithiasis are involved in local immunological responses through the transport of secretory component and IgA into bile (137). Human cholangiocytes also express ICAM-1 (26, 48), lymphocyte function-associated antigen (LFA)-3 (48), CD40 (48), and Fas (137). Recent studies in rats showed that secretin increases ductal secretion and that secretin stimulation of Cl⁻/HCO₃⁻ exchanger activity was independent of the increase in the second messengers cAMP, cGMP, and cytosolic Ca²⁺ (44, 46). Secretin-stimulated biliary secretion is dependent on anion exchangers, Cl⁻ and K⁺ channels, and carbonic anhydrase but not microtubules (46), through mechanisms different from those established for secretin (6, 9–18, 22, 38, 62, 63, 83–87, 98, 143).
and human lymphocyte antigen (HLA) class 1 (26, 48). Human cholangiocytes secrete interleukin (IL)-6 and tumor necrosis factor-α in bile (158). Furthermore, we showed previously (4) that interferon-γ inhibits SR gene expression and secretin-stimulated bicarbonate-rich choleresis in a murine model of cirrhosis induced by chronic CCl₄ treatment. This observation suggests that cytokines produced by cholangiocytes in an autocrine/paracrine manner may regulate ductal secretion. Moreover, we recently showed (99) that human biliary cell lines and murine and rat cholangiocytes express IL-5 receptors and that IL-5 inhibits Cl⁻ channel currents.

HETEROGENEITY OF SECRETIN-INDUCED CHOLANGIOCYTE RESPONSES

Recently, the isolation and phenotypic characterization of distinct subpopulations of small (~8 µm in size) and large (~13 µm in size) cholangiocytes (13, 14) and small (<15 µm in external diameter) and large (>15 µm in external diameter) IBDU (6) allowed us to demonstrate that the intrahepatic biliary epithelium is morphologically and functionally heterogeneous (5, 6, 8, 13, 14, 85, 86). These studies showed that the SR is solely expressed by large cholangiocytes in large ducts (6, 13), which respond physiologically to secretin with increases in cAMP levels, Cl⁻ efflux, and Cl⁻/HCO₃⁻ exchanger activity (see Fig. 4). Other studies showed that secretin-stimulated ductal secretion of large cholangiocytes is inhibited by somatostatin, whose receptors (i.e., SSTR₂) are expressed by large but not small cholangiocytes (9). Recent studies demonstrated that ET-1 decreases the secretin-induced secretion of large cholangiocytes by selectively interacting with ETA receptors (38). Consistent with this concept, other studies showed that the Cl⁻/HCO₃⁻ exchanger [an important component of secretin-stimulated bicarbonate-rich choleresis (12, 13, 16, 18, 84, 119, 134)] is only expressed by large bile ducts in humans (96).

SUMMARY AND FUTURE PERSPECTIVES

The findings discussed in this review emphasize that secretin-stimulated ductal bile secretion is cooperatively regulated by a number of factors, some with stimulatory effects [e.g., VIP, ACh, the bile acids TC and TLC (5, 7, 16)] and some with inhibitory action [e.g., somatostatin, gastrin, ET-1, AP (9, 17, 38, 63, 143)]. The recent data related to the role and mechanisms of action of ACh (16) and bile acids (5, 7) are the most interesting findings related to the modulation of secretin-stimulated ductal secretion. The findings that rat cholangiocytes of different sizes differentially re-

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**Fig. 4.** Schematic representation of the secretory heterogeneity of the intrahepatic biliary tree. The cartoon shows that the major sites of secretin- and somatostatin-regulated transport of water and electrolytes involve cholangiocytes in large bile ducts (which contain secretin and somatostatin receptors), whereas small cholangiocytes in small ducts do not express secretin and somatostatin receptors and do not participate in hormone-regulated ductal secretion.
spend to liver injury and/or toxins has clinical relevance because a number of chronic cholestatic liver diseases (e.g., PBC and PSC) are characterized by a spotty rather than a diffuse proliferative response (12, 120). Studies are needed to evaluate the role and mechanisms of action of the adrenergic and dopaminergic nerves in the regulation of secretin-stimulated bicarbonate-rich choleresis. Taking into account that after BDL microvascular proliferation occurs only adjacent to large proliferating ducts (59) and that secretin-stimulated secretion is only present in large cholangiocytes (9, 13, 14, 85, 86), further studies are necessary to understand the role of blood supply and vascularly derived factors in the regulation of secretin-stimulated ductal bile secretion. On the basis of these findings, it seems likely that our understanding of secretin stimulation of ductal secretory functions will continue to grow as a focus of increasing attention and importance.

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