Hormonal Regulation of Bicarbonate Secretion in the Biliary Epithelium

Domenico Alvaro,a Alessandro Giglioizzi, Flavia Fraioli, Rosamaria Romeo, Emanuela Papa, Marco Delle Monache, and Livio Capocaccia

II Division of Gastroenterology, Department of Clinical Medicine, University of Rome "La Sapienza," Rome Italy

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Bicarbonate excretion in bile is a major function of the biliary epithelium. It is driven by the apically located Cl-/HCO₃⁻ exchanger which is functionally coupled with a cAMP-dependent Cl⁻ channel (CFTR). A number of hormones and/or neuropeptides with different mechanisms and at different intracellular levels regulate, in concert, the processes underlying bicarbonate excretion in the biliary epithelium. Secretin induces a bicarbonate rich cholerisis by stimulating the activity of the Cl-/HCO₃⁻ exchanger by cAMP and protein kinase A mediated phosphorylation of CFTR regulatory domain. Protein phosphatase 1/2A are involved in the run-down of secretory stimulus after secretin removal. Acetylcholine potentiates secretin-cholerisis by inducing a Ca²⁺-calcineurin mediated "sensitization" of adenyl cyclase to secretin. Bombesin and vasoactive intestinal peptide also enhance the Cl-/HCO₃⁻ exchanger activity, but the intracellular signal transduction pathway has not yet been defined. Somatostatin and gastrin inhibit basal and/or secretin-stimulated bicarbonate excretion by down-regulating the secretin receptor and decreasing cAMP intracellular levels induced by secretin.

INTRODUCTION

The intrahepatic biliary tree comprises a network of interconnecting ducts, of increasing diameter, from the duct of Hering to the extrahepatic bile ducts, which are lined by typical epithelial cells – the cholangiocytes – representing 3-5 percent of the total nuclear population in the liver [1-5]. Thought for a long time to play only a passive function of transporting bile into the duodenum, the biliary epithelium is now the focus of extensive investigation, highlighting extraordinary properties in terms of secretion, absorption, proliferation and signaling toward the other parenchymal and mesenchymal liver cells [1-5]. The biliary tree is nourished by the peribiliary vascular plexus [6], which stems from the hepatic artery branches and flows into the hepatic sinusoids. The peribiliary vascular plexus plays a fundamental role in supporting the secretory and absorptive functions of the biliary epithelium. In the light of its anatomical and functional condition, the biliary tree represents, within the liver, a barrier between bile and blood, the functional regulation of which may play an important role in conditioning the physio-pathology of the liver. On the one hand, hepatocytes may influence the function of biliary epithelium through the secretion in bile of substances (bile salts, purines), which have been recently demonstrated to

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a To whom all correspondence should be addressed: Domenico Alvaro, M.D., Via Valsolda 45/i, 00141 Rome, Italy. Fax: 39-6-4440806; E-mail: alvaro@axrma.uniroma1.it.

b Abbreviations: BDE, bile duct epithelial (cells); DIDS, 4,4'-disothiocyanatostilbene-2,2'-disulfonic acid; CFTR, cystic fibrosis transmembrane conductance regulator; PKA, protein kinase A; PKC, protein kinase C; IBDU, isolated bile duct unit; VIP, vasoactive intestinal peptide; RT-PCR, reverse transcriptase polymerase chain reaction; Rp-cAMPS, Rp-adenosine 3'-5' monophosphorothioate; Sp-cAMPS, Sp-adenosine 3'-5' monophosphorothioate; PP, protein phosphatase; ANIT, α-naphthyl-isothiocyanate.
regulate the biology of the biliary epithelium [7, 8]. On the other hand, the biliary epithelium may reabsorb from bile or secrete into the peribiliary plexus substances, which may influence hepatic circulation (endothelin), proliferation of parenchymal and mesenchymal cells (growth factors), inflammation and fibrosis (cytokines) [9].

ELECTROLYTE TRANSPORT PROCESSES IN THE BILIARY EPITHELIUM

The biliary epithelium plays a major role in determining the final composition of bile reaching the duodenum. Within large interspecies differences, ductal bile may account for up to 40 percent of the total bile flow but, most importantly, the composition of bile flowing in the biliary tree is markedly modified by secretory and absorptive processes taking place in cholangiocytes [1-5]. Cholangiocytes possess a number of transporters which affect electrolyte exchange between bile and plasma (Figure 1). In the last few years, thanks to newly developed experimental models (primary cultures of cholangiocytes, cell lines, bile duct units), major progress has been made in identifying and characterizing electrolyte transport processes in cholangiocytes. In bile duct epithelial cells (BDE) isolated from bile duct ligated or normal rat liver, the H+/HCO₃⁻ transport processes involved in the regulation of intracellular pH (pHᵢ) have been identified using fluorescent dyes [10-
Two acid extruders, the Na+/H+ exchanger (electroneutral) and Na+/HCO$_3^-$ symporter (electrogenic) drive the recovery of pH$_1$ from acid load, while a Na$^+$-independent Cl$^-$/HCO$_3^-$ exchanger (electroneutral) is responsible for HCO$_3^-$ excretion [10-12]. The latter, thought to be the main mechanism of bicarbonate excretion in bicarbonate secretory epithelia, has been also functionally identified in isolated human cholangiocytes [13] and biliary cell lines [14] and, immunohistochemically, in the apical pole of human liver cholangiocytes [15]. In human isolated cholangiocytes and in human biliary cell lines [13, 14], an electroneutral Na$^+$-dependent Cl$^-$/HCO$_3^-$ exchanger instead of the electrogenic Na$^+/$/HCO$_3^-$ symporter seems to be involved in HCO$_3^-$ loading into the cell. Using electrophysiological techniques, channel activity and conductance pathways have been defined. In isolated rat cholangiocytes, a low conductance cAMP-activated Cl$^-$ channel with a linear current-voltage relationship, time independence and insensitivity to DIDS has been described [16]. These electrical properties are similar to those associated with the cystic fibrosis transmembrane conductance regulator (CFTR). Immunofluorescence and immunocytochemistry studies have identified CFTR in the apical pole of rat cholangiocytes and human cholangiocytes [16-18]. As in other bicarbonate secretory epithelia, a close functional cooperation between CFTR and the Cl$^-$/HCO$_3^-$ exchanger could play a key role in the regulation of bicarbonate secretion by hormones acting via the cAMP-protein kinase A (PKA) pathway. A Ca$^{2+}$-dependent Cl$^-$ conductance showing outward rectification of the current-voltage relation, time-dependence and DIDS sensitivity as well as K$^+$ conductance has been identified in rat cholangiocytes and human biliary cell lines [18-20]. Finally, cAMP and Ca$^{2+}$-insensitive high-conductance Cl$^-$ channels, inhibited by pertussis toxin-sensitive heterotrimeric GTP-binding proteins, have also been described in isolated rat cholangiocytes although their physiological relevance is still unknown [20]. Cl$^-$ entrance seems to be mainly driven by the Na$^+/K^+$/2Cl$^-$ co-transporter recently identified in rat isolated bile duct units (IBDU) [5].

**BILIARY BICARBONATE SECRETION**

Bicarbonate secretion in bile is a major function of the biliary epithelium. It contributes significantly to the total bicarbonate need for digestive functions, is a major determinant of alkalinity and hydration of hepatic bile and represents one of the most important buffer systems. In addition, by decreasing the amount of protonated moiety, it conditions the passive absorption of lipophilic weak acid through the biliary epithelium. Bicarbonate secretion in bile is driven by the activity of the apically located Cl$^-$/HCO$_3^-$ exchanger and it is now known to be regulated by different hormones and neuropeptides [21]. Secretin [10, 11], bombesin [22] and vasoactive intestinal peptide (VIP) [23] induce ductal bicarbonate rich choleresis by stimulating the activity of the Cl$^-$/HCO$_3^-$ exchanger. Acetylcholine potentiates [24] the secretin stimulatory effect on the Cl$^-$/HCO$_3^-$ exchanger, while somatostatin [25] and gastrin [26] counter-regulate basal and/or secretin induced bicarbonate excretion. Investigation into the signal transduction pathways involved in the regulation of bicarbonate excretion by different hormones or neuropeptides is still at an early stage. As far as secretin is concerned, however, significant progress has been made [27]. Thanks to this progress, it now emerges that a number of intracellular mechanisms, at different levels from the initial step of receptor activation to the final step of anion exchanger stimulation, are involved in the regulation of bicarbonate excretion in bile (Figure 2).
REGULATION OF BICARBONATE EXCRETION AT RECEPTOR LEVEL

Secretin receptor was the first hormone receptor identified in cholangiocytes initially by autoradiographic techniques [28] and, more recently, at a molecular level [1, 2]. Secretin receptors are up-regulated at transcriptional level during cholangiocyte proliferation (bile duct ligation, heptectomy), in vitro exposure to bile salts, in vivo administration of secretin in the rat and finally in the model of CCl4-induced liver cirrhosis [1, 2, 8]. On the contrary, secretin receptors are down-regulated by gastrin [26]. In other cell types, secretin receptors may be also modulated by VIP, which induces in IBDU a dramatic
bicarbonate rich fluid secretion, even greater than that induced by secretin [21, 23]. Whether VIP also interacts in cholangiocytes with secretin receptor is as yet unknown.

After binding its own receptor, secretin induces an intracellular cAMP increase, while cGMP and Ca++ levels are unaffected [1, 2]. The mechanism by which the activation of secretin receptor induces an increase of cAMP intracellular levels, probably involves a Gs-protein-mediated induction of adenyl cyclase. This aspect, however, has not yet been explored in cholangiocytes.

REGULATION OF BICARBONATE EXCRETION
AT THE LEVEL OF ADENYL CYCLASE

Secretin is the only hormone demonstrated to date which stimulates adenyl cyclase and so induces cAMP increase and cAMP-mediated stimulation of the anion exchanger. However, at least three hormones or neuropeptides namely, acetylcholine, somatostatin and gastrin, may each influence, differently, bicarbonate biliary excretion by regulating adenyl cyclase activity [21]. Acetylcholine binds muscarinic M3 subtype receptors, as recently demonstrated by immunohistochemistry, immunoelectron-microscopy and RT-PCR in IBDU and isolated rat cholangiocytes [24]. M3 receptor activation results in the increase of intracellular Ca++ due to both mobilization of intracellular Ca++ stores and influx of extracellular Ca++ [29]. The acetylcholine-induced Ca++ increase leads to a "sensitization" of adenyl cyclase to secretin, which doubles cAMP intracellular levels and finally results in an almost maximal stimulation of the Cl-/HCO3− exchanger. By this mechanism of adenyl cyclase modulation, acetylcholine does not affect basal fluid secretion [24, 29], but markedly potentiates the secretin choleric effect [24]. Since secretin targets cholangiocytes during parasympathetic predominance (digestive phase), the coordinated regulation of bicarbonate excretion by secretin (cAMP) and acetylcholine (Ca+++) could serve as a means of amplifying the secretory response just when the bicarbonate requirement in the intestine is maximal [24]. Somatostatin acts also at the level of adenyl cyclase but, contrary to acetylcholine, it exerts inhibitory effects [27]. Somatostatin binds SSTR2 subtype receptors which have been detected by RNase protection assay in rat and human cholangiocytes [27], SSTR2 receptors, in many different cell types, are linked with a regulation of K+ and Ca++ channels [21], but whether somatostatin affects electrolyte transport processes in cholangiocytes has not yet been investigated. Rather, by acting exclusively on large bile ducts, somatostatin inhibits basal and secretin-stimulated ductal flow, the latter by inhibiting secretin induced cAMP intracellular levels which probably occurs via Gi-protein mediated inhibition of adenyl cyclase activity. Similar to somatostatin, gastrin also inhibits secretin cholestasis by blocking cAMP formation through a mechanism which probably involves a Gi-protein mediated inhibition of adenyl cyclase. In contrast to somatostatin, gastrin does not affect basal bile flow [26].

Apart from hormonal regulation, two bile salts, taurocholate and taurolithocholate, enhance secretin cholestasis, through a marked increase of secretin induced cAMP intracellular level and a consequent potentiation of secretin stimulation of the Cl-/HCO3− exchanger [8].

REGULATION OF BICARBONATE SECRETION AT THE LEVEL OF CFTR

Once cAMP intracellular levels are increased in response to secretin, the recruitment and activation of PKA is a next, critical step before the stimulation of bicarbonate extrusion. We recently demonstrated the key involvement of PKA in the secretin-induced fluid secretion and Cl-/HCO3− exchanger activity in IBDU, by using two diastereomers of an
analogue of natural cAMP, Rp-cAMPS and Sp-cAMPS [27]. The two diastereomers are characterized by marked stability and membrane permeability, as well as by high resistance to degradation by phosphodiesterases. However, while Sp-cAMPS mimics the effect of natural cAMP, binding PKA, dissociating the catalytic subunit and thereby activating PKA, the R-diastereomer (i.e. Rp-cAMPS) binds to the regulatory subunit of PKA, but does not dissociate the catalytic subunit and thus, acts as a competitive inhibitor for PKA activators. We first demonstrated that the Sp-cAMPS diastereomer is able to reproduce the effect of secretin by stimulating the Cl-/HCO3- exchanger and, as a consequence, alkaline fluid secretion in the lumen of IBDU. We thereby demonstrated that when PKA activity was blocked with the competitive inhibitor, Rp-cAMPS, secretin failed to induce any significant stimulation of fluid secretion or of Cl-/HCO3- exchanger activity [27]. In cholangiocytes, CFTR activation is a prerequisite for secretin stimulation of the Cl-/HCO3- exchanger [10-12]. Thus, our findings indicate that phosphorylation of CFTR by PKA is an essential step in the mechanism of action of secretin. A similar effect of Rp-cAMPS has been found in rat hepatocyte coupled [30], where it blocks the stimulation of the Cl-/HCO3- exchanger induced by glucagon, a hormone from the same family as secretin, which induces a bicarbonate-rich cholerasis of hepatocyte origin. In hepatocyte, however, CFTR is absent and, thus, the target of PKA phosphorylation is unknown. The precise relationship existing between CFTR activation by cAMP-PKA and Cl-/HCO3- exchanger stimulation is still being debated [10-12]. It has been postulated that the opening of Cl- channels induces an increased out to in Cl- gradient favoring the Cl-/HCO3- exchange mechanism [1, 2]. We have proposed [10], in alternative, that the depolarization induced by the Cl- channel opening, enhances the entrance of bicarbonate via activation of the electrogenic Na+HCO3- symporter. This in turn stimulates the Cl-/HCO3- exchanger at its HCO3- or pH sensitive site. Whatever the mechanism involved, activation of CFTR is a prerequisite for stimulation of the Cl-/HCO3- exchanger by secretin and the our data indicates that PKA phosphorylation of CFTR is essential for secretin stimulation of the Cl-/HCO3- exchanger and ductal fluid secretion. Current knowledge on the relationship between channel activity and phosphorylation status is not complete. It has been shown that the regulatory domain of CFTR contains multiple PKA phosphorylation consensus sequences [31-34]. In vivo studies have implicated at least four serine residues in the R domain of CFTR, the phosphorylation of which by PKA is crucial for regulated Cl- transport [31-34]. Mutation of these serine residues, taken together but not singly, prevented cAMP dependent activation of CFTR dependent Cl- conductance [31-34]. The conceptual model, similar to K+ channel gating [33], postulates that the R domain occludes the conduction pathway and that phosphorylation by PKA induces conformational change allowing Cl- conductance [31-34]. Phosphorylation consensus sequences for protein kinase C (PKC) and Ca++-calmodulin kinases have also been demonstrated in the R domain of CFTR, expressed in different cell types, but their role in the regulation of channel activity is currently disputed [31]. In some studies concerning different types of cells expressing CFTR, channel activity stimulated by PKC alone is much smaller than that generated by PKA [31]. When combined with PKA, the additional PKC-induced phosphorylation may greatly potentiate the activity of CFTR [31]. Other studies, however, have failed to confirm a synergism in the activation of CFTR by PKA and PKC [31]. Thus the effect of PKC activation on the regulation of cAMP-dependent Cl- channels is currently controversial. We examined the involvement of PKC in the secretin signal transduction pathway by using the phorbol ester, PMA, as PKC agonist, and staurosporine as PKC antagonist [27]. We found that induction of PKC with phorbol ester was unable to affect fluid secretion and Cl-/HCO3- activity in IBDU and, in addition, blocking PKC with staurosporine failed to influence the secretin effect. Thus, the secretin-induced bicarbonate-rich cholerasis does not seem to involve PKC.
Once CFTR is activated by cAMP-PKA dependent phosphorylation, the run-down of the secretory processes occurs by a dephosphorylation step driven by endogenous protein phosphatases (PP). This conclusion was reached by showing that exposure of IBDU to okadaic acid (inhibitor of serine/threonine PP-1 and PP-2A) results in persistence of secretion even after removal of secretin, while in controls, removal of secretin results in a return to basal state of both fluid secretion and Cl⁻/HCO₃⁻ activity [27]. With excised patches of membranes from NIH 3T3 fibroblasts stably expressing CFTR, PP-2A, but not PP-1 nor PP-2B, is able to completely dephosphorylate and thus inactivate CFTR [34]. In cardiac CFTR Cl⁻ channels, PP-1 and PP-2A are absolutely necessary (okadaic acid sensitivity) for full CFTR dephosphorylation after agonist (isoproterenol, forskolin) induced activation, but other phosphatases can also partially dephosphorylate the channel [31]. Although we cannot exclude that other phosphatases are involved, the PP-1 and PP-2A are good candidates for CFTR dephosphorylation and inactivation in the biliary epithelium.

Alkaline phosphatase is another protein phosphatase (non-specific) which inhibits the CFTR activity in the biliary cell line [35] as well as in other cell types [34] expressing CFTR. Since cholangiocytes are continuously exposed in their apical pole to high concentration of alkaline phosphatase and the serum levels of this enzyme specifically increase during cholestasis, the role of alkaline phosphatase in controlling secretory processes in physiology or during different pathologic conditions should merit major attention.

REGULATION OF BICARBONATE EXCRETION BY VESICLE TARGETING OF CFTR OR Cl⁻/HCO₃⁻ EXCHANGER TO THE APICAL MEMBRANE

Bicarbonate secretion in bile and its hormonal regulation could also depend on the number of transport proteins (Cl⁻/HCO₃⁻) or channels (CFTR) targeted from the endocellular pool to the apical membrane as well as on the regulation of their recycling. In this regard, CFTR could play a relevant role. In fact, other than as a Cl⁻ channel, CFTR, has been shown to play a role in controlling intracellular vesicle trafficking in different cell types [31]. Activation of the intracellular pool of CFTR by PKA could regulate vesicle acidification and by this mechanism could influence the amount of CFTR and of other membrane proteins, such as the Cl⁻/HCO₃⁻ exchanger in the apical membrane of bile duct epithelial cells, thus influencing response to secretin. In cells expressing CFTR, cAMP-PKA seems to act by blocking the retrieval of CFTR from the apical membrane to the intracellular pool while Ca²⁺ operate by favoring vesicle-mediated insertion of CFTR into the apical membrane [31].

Unfortunately, research in this field and in general on vesicle trafficking in cholangiocytes is only at an early stage. Preliminary data from Benedetti et al. [36] suggested that transcytosis of lucifer yellow and/or horseradish peroxidase in rat cholangiocytes is sensitive to colchicine (microtubule inhibitor), brefeldin A (an inducer of retrieval of Golgi to endoplasmic reticulum) and wortmannin (phosphoinositol-3-kinase inhibitor). Kato et al. [37] reported a colchicine inhibition of the secretin-stimulated acridine orange exocytosis in isolated cholangiocytes. In contrast, Dallenbach and Renner [38] found that secretin choleresis in rats with ANIT-induced cholangiocyte hyperplasia was unaffected by colchicine. Thus, whether CFTR activation by PKA could also influence the rate of secretin response in IBDU by regulating vesicle trafficking cannot be defined at this stage.
REGULATION OF BICARBONATE EXTRUSION BY Ca++-DEPENDENT PATHWAYS

Ca++ agonists can induce Cl– efflux in cholangiocytes by acting on Ca++ or Ca++ calmodulin dependent Cl– channels [16-20, 39]. In addition, Ca++ agonists could induce a marked Cl– efflux as the effect of intracellular potential increase due to the opening of the Ca++-dependent K+ channel. Nevertheless, there is no current evidence that hormones, acting via Ca++ pathways, are capable of stimulating bicarbonate excretion. Acetylcholine or carbachol, for example, failed to induce bicarbonate extrusion and to stimulate ductal bile flow in IBDU [24, 29]. If the activity of the Cl–/HCO3– exchanger is stimulated as a consequence of the Cl– gradient, an enhancement of bicarbonate excretion via this anion exchanger should occur, regardless of whether Cl– efflux is induced via Ca++ channel or CFTR activation. A possible explanation could be that in cholangiocytes the number and density of cAMP-dependent Cl– channels is markedly higher than the number of Ca++ channels and, as a consequence, Cl– efflux stimulated by cAMP pathways is markedly higher and time persistent while Ca++ stimulated Cl– efflux is lower and transient. Since, apart from hormones, intracellular Ca++ increase may be induced by ATP and bile salts, the effect of Ca++ pathways, on Cl– and K+ conductance, bicarbonate excretion, vesicle trafficking and adenyl cyclase regulation should be further investigated.

SUMMARY AND CONCLUSIONS

Although research on the physiology and biology of biliary epithelium only began in the last decade, an impressive mass of work has been performed. These studies clearly demonstrate that the biliary epithelium possess extraordinary properties in term of secretion, absorption, proliferation and signaling toward other parenchymal and mesenchymal cells in the liver. Major progress has been made in the field of hormonal regulation of biliary bicarbonate secretion that is one of the major functions of the biliary epithelium. Several hormones or neuropeptides act in the regulation of this process with different mechanisms operating at the level of receptors, adenyl cyclase, vesicle trafficking as well as electrolyte channels or exchangers located in the apical membrane. The signal transduction pathway has been particularly investigated for secretin, demonstrating that activation/inactivation of CFTR by phosphorylation/dephosphorylation processes operated by PKA and PP-1/2A, is the crucial step underlying secretin choleresis.

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REFERENCES

1. Alpini, G., Phillips, I.O., and LaRusso, N.F. The biology of biliary epithelia. In: Arias, I.M., Fausto, N., Jacoby, W.B., Schachter, D.A., and Shafritz, D.A., eds. The Liver: Biology and Pathobiology. New York: Raven Press, Ltd. 1994, pp. 623-653.
2. Roberts, S.K. and LaRusso, N. Pathobiology of biliary epithelia. Curr. Opin. Gastroenterology 10:526-533, 1994.
3. Boyer, J.L. Bile duct epithelium: frontiers in transport physiology. Am. J. Physiol. 270:G1-G5, 1996.
4. Desmet, V.J. Anatomy, development and pathology of the biliary tree. In: Alvaro, D., Benedetti, A., and Strazzabosco, M., eds. Vanishing Bile Duct Syndrome – Pathophysiology. Kluwer Ac. Publishers, London; 1997, pp. 5-12.
5. Boyer, J.L. Vanishing Bile Duct Syndrome-from bench to bed side. In: Alvaro, D., Benedetti, A., and Strazzabosco, M., eds. Vanishing Bile Duct Syndrome – Pathophysiology. Kluwer Ac. Publishers, London; 1997. pp. 240-246.
6. Gaudio, E., Onori, P., Pannarale, L., and Alvaro, D. Hepatic microcirculation and peribiliary plexus in experimental biliary cirrhosis: a morphological study. Gastroenterology 111:1118-1124, 1996.

7. Fitz, J.G. Evidence for paracrine regulation of biliary Cl⁻ secretion by purinergic signaling. In: Alvaro, D., Benedetti, A., and Strazzabosco, M., eds. Vanishing Bile Duct Syndrome-Pathophysiology. Kluwer Ac. Publishers, London; 1997. pp. 65-71.

8. Alpini, G., Glasier, S., Phinizy, J., Lasater, J., Robertson, W., Rodgers, R., and LeSage, G. Isolation and characterization of specific cholangiocyte subpopulations involved in bile acid regulated ductal bile secretion and proliferation. Hepatology 24:1 47A, 1 996.

9. Strazzabosco, M., Spirito, C., Zsembry, A., Granato, A., Fabris, L., Cavese, M., Iemomolo, R.M., Okolicsanyi, L., and Crepaldi, G. Pathophysiology of the biliary epithelium. In: Alvaro, D., Benedetti, A., Strazzabosco, M., eds. Vanishing Bile Duct Syndrome-Pathophysiology. Kluwer Ac. Publishers, London; 1997. pp. 117-127.

10. Alvaro, D., Cho, W.K., Mennone, A., and Boyer, J.L. Effect of secretin on intracellular pH regulation in isolated rat bile duct epithelial cells. J. Clin. Invest. 92:1314-1325, 1993.

11. Mennone, A., Alvaro, D., Cho, W., and Boyer, J.L. Isolation of small polarized bile duct units. Proc. Natl. Acad. Sci. USA 92:6527-6531, 1995.

12. Alvaro, D., Mennone, A., and Boyer, J.L. Effect of ursodeoxycholic acid on Intracellular pH regulation in isolated rat bile duct epithelial cells. Am. J. Physiol. 265:G783-G791, 1993.

13. Strazzabosco, M., Joplin, R., Zembry, A., Granato, A., Spirito, C., Poci, C., and Crepaldi, G. Bicarbonate transport in cultured human intrahepatic bile duct epithelial cells (hBDE) from normal subjects and patients with primary biliary cirrhosis (PBC). Hepatology 2:404A, 1994.

14. Strazzabosco, M., Poci, C., Spirito, C., Sartori, L., Knuth, A., Crepaldi, G. Effect of ursodeoxycholic acid on intracellular pH in a bile duct epithelium-like cell line. Hepatology 19:145-154, 1994.

15. Martinez-Anso, E., Castillo, J.E., Diez, J., Medina, J.F., and Prieto, J. Immunohistochemical detection of chloride/bicarbonate anion exchanger in human liver. Hepatology 19:1400-1406, 1994.

16. Fitz, J.G., Basavappa, S., McGill, J., Melhus, O., and Cohn, J.A. Regulation of membrane chloride currents in rat bile duct epithelial cells. J. Clin. Invest. 91:319-328, 1993.

17. Cohn, J.A., Strong, T.V., Picciotto, M.R., Nairn, A.C., Collins, F.S., and Fitz, J.G. Localization of the cystic fibrosis transmembrane conductance regulator in human bile duct epithelial cells. Gastroenterology 105:1857-1864, 1993.

18. Basavappa, S., Middleton, J., Mangel, A.W., McGill, J.M., Cohn, J.A., and Fitz, J.G. Cl⁻ and K⁺ transport in human biliary cell lines. Gastroenterology 104:1796-1805, 1993.

19. McGill, J.M., Basavappa, S., and Fitz, J.G. Characterization of high-conductance anion channels in rat bile duct epithelial cells. Am. J. Physiol. 262:G703-G710, 1992.

20. McGill, J.M., Gettys, W., Basavappa, S., and Fitz, J.G. GTP-binding proteins regulate high conductance anion channels in rat bile duct epithelial cells. J. Membr. Biol. 133:253-261, 1993.

21. Alvaro, D., Gigliozzi A., La Rosa T., Crali L., Furfaro S., Fraioli F., Francia C., Romeo, R., and Capocaccia, L. In: Alvaro, D., Benedetti, A., and Strazzabosco, M., eds. Vanishing Bile Duct Syndrome-Pathophysiology. Kluwer Ac. Publishers, London; 1997. pp. 52-64.

22. Cho, W.K., Mennone, A., and Boyer, J.L. Effect of bombesin on secretion in isolated polarized intrahepatic bile ductular units (IBDU) (Abstract). Gastroenterology 108:A1049, 1995.

23. Cho, W.K., Mennone, A., Rydberg, S.A., and Boyer, J.L. VIP is a potent stimulus of bicarbonate and fluid secretion in bile ducts. (Abstract). Hepatology 22:A294, 1995.

24. Alvaro, D., Alpini, G., Jezequel, A.M., Bassotti, C., Francia, C., Fraioli, F., Romeo R., Marucci L., Le Sage, G., Glasier S.S., and Benedetti A. Role and mechanisms of action of acetycholine in the regulation of cholangiocyte secretory functions. J. Clin. Invest. 100:1349-1362, 1997.

25. Tietz, P.S., Alpini, G., Pham, L.D., and LaRusso, N.F. Somatostatin inhibits secretin-induced ductal hyperplasia and exocytosis by cholangiocytes. Am. J. Physiol. 269:G110-G118, 1995.

26. Glasier, S., Rodgers, R., Phinizy, J.L., Robertson, W., Lasater, J., Caligiuri, A., Tretjak, Z., LeSage, G., and Alpini, G. Gastrin inhibits secretin-induced ductal secretion by interaction with specific receptors on rat cholangiocytes. Am. J. Physiol. 273:G1061-1070, 1997.

27. Alvaro, D., Mennone, A., and Boyer, J.L. Role of protein kinases and phosphatases in the regulation of fluid secretion and Cl⁻/HCO₃⁻ exchange in cholangiocytes Am. J. Physiol. 273:G303-G313, 1997.

28. Alvaro, D., Della Guardia, P., Bini, A., Gigliozzi, A., Furfaro, S., La Rosa, T, Piat, C., and Capocaccia, L. Effect of glucagon on intracellular pH regulation in isolated rat hepatocyte couples. J. Clin. Invest. 96:665-674, 1995.
29. Farouk, M., Vigna, S., McVey, D.C., and Meyers, W.C. Localization and characterization of secretin binding sites expressed by rat bile duct epithelium. Gastroenterology 102:963-968, 1992.
30. Nathanson, M.H., Burgstahler, A.D., Mennone, A., and Boyer, J.L. Characterization of cytosolic Ca^{2+} signaling in rat bile duct epithelia. Am. J. Physiol. 271:G86-G96, 1996.
31. Frizzel, R.A. and Morris, A.P. Chloride conductance of salt-secreting epithelial cells. Curr. Top. Memb. 42:173-214, 1994.
32. Cheng, S.H., Rich, D.P., Marshall, J., Gregory, R.J., Welsh, M.J., and Smith, A.E. Phosphorylation of the R-domain by cAMP dependent protein kinase regulates the CFTR chloride channel. Cell 66:1027-1036, 1991.
33. Hoshi, T., Zagotta, W.N., and Aldrich, R.W. Biophysical and molecular mechanisms of Shaker potassium channel inactivation. Science 250:533-538, 1990.
34. Berger, H.A., Travis, S.M., and Welsh, M.J. Regulation of the cystic fibrosis transmembrane regulator Cl^{-} channel by specific protein kinases and protein phosphatases. J. Biol. Chem. 268:2037-2047, 1993.
35. McGill, J.M., Yen, M.S., and Kwiatkowski, A.P. Alkaline phosphatase inhibits biliary epithelial cell chloride channels. Gastroenterology 110:1264A, 1996.
36. Benedetti, A. Transport of non-electrolyte biliary components in cholangiocytes. In: Alvaro, D., Benedetti, A., and Strazzabosco, M., eds. Vanishing Bile Duct Syndrome - Pathophysiology. Kluwer Ac. Publishers, London; 1997, pp. 72-81.
37. Kato, A., Gores, G.J., and LaRusso, N.F. Secretin stimulates exocytosis in isolated bile duct epithelial cells by a cyclic AMP-mediated mechanism. J. Biol. Chem. 267:15523-15529, 1992.
38. Dallenbach, A. and Renner, E.L. Colchicine does not inhibit secretin-induced choleresis in rats exhibiting hyperplasia of bile ductules: evidence against a pivotal role of exocytic vesicle insertion. J. Hepatology 22:338-348, 1995.
39. McGill, J.M., Yen, M.S., Basavappa, S., Mangel, A.W., and Kwiatkowski, A.P. ATP-activated chloride permeability in biliary epithelial cells is regulated by calmodulin-dependent protein kinase II. Biochem. Biophy. Res. Commun. 208:457-462, 1995.