

TOPIC HIGHLIGHT

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Cholangiocyte anion exchange and biliary bicarbonate excretion

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Abstract

Primary canalicular bile undergoes a process of fluidization and alkalization along the biliary tract that is influenced by several factors including hormones, innervation/neuropeptides, and biliary constituents. The excretion of bicarbonate at both the canaliculi and the bile ducts is an important contributor to the generation of the so-called bile-salt independent flow. Bicarbonate is secreted from hepatocytes and cholangiocytes through parallel mechanisms which involve chloride efflux through activation of Cl⁻ channels, and further bicarbonate secretion *via* AE2/SLC4A2-mediated Cl⁻/HCO₃⁻ exchange. Glucagon and secretin are two relevant hormones which seem to act very similarly in their target cells (hepatocytes for the former and cholangiocytes for the latter). These hormones interact with their specific G protein-coupled receptors, causing increases in intracellular levels of cAMP and activation of cAMP-dependent Cl⁻ and HCO₃⁻ secretory mechanisms. Both hepatocytes and cholangiocytes appear to have cAMP-responsive intracellular vesicles in which AE2/SLC4A2 colocalizes with cell specific Cl⁻ channels (CFTR in cholangiocytes and not yet determined in hepatocytes) and aquaporins (AQP8 in hepatocytes and AQP1 in cholangiocytes). cAMP-induced coordinated trafficking of these vesicles to either canalicular or cholangiocyte luminal membranes and further exocytosis results in increased osmotic forces and passive movement of water with net bicarbonate-rich hydrocholeresis.

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Key words: AE2 anion exchanger; Bile salt-independent flow; Biliary bicarbonate excretion; Regulation of

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INTRODUCTION

Hepatocytes and cholangiocytes are the epithelial cells in the liver, and they both participate in the production of bile. The hepatocytes, the major liver cell population (65%), generate the primary bile at their canaliculi^[1]. As an important complement, the epithelial cells lining intrahepatic bile ducts or cholangiocytes (5% of the liver cell population) exert a series of reabsorptive and secretory processes which dilute and alkalize the bile flow during its passage along the biliary tract^[2-6]. Modifications of ductal bile appear to be tightly regulated by the action of nerves, biliary constituents, and some peptide hormones like secretin. Accordingly, it is possible to distinguish between three different bile flow fractions: (1) the canalicular bile salt-dependent flow (30% to 60% of spontaneous basal bile flow) that is driven by concentrative secretion of bile acids by the hepatocytes followed by a facilitated efflux of water^[7,8]; (2) the canalicular bile salt-independent flow (another 30% to 60% of spontaneous basal bile flow)^[1,8-10], which is also created by hepatocytes but through active secretion of both inorganic and organic compounds (mainly bicarbonate^[11,12] and glutathione^[13], respectively); and (3) the ductal bile flow, that is the bile salt-independent flow fraction modified and contributed by cholangiocytes, mainly through production of a bicarbonate-rich fluid in response to secretin^[2,14-17] and other regulatory factors^[2,5,6,17,18].

In the last decade, the use of molecular- and cell-biology tools and the availability of suitable experimental models have greatly facilitated our knowledge on the processes involved in bile generation and modification. This review summarizes some of the experimental models employed in biliary studies, and focuses on the biliary excretion of bicarbonate, the chief factor responsible for ductal bile alkalization and fluidization, and the role and interactions of regulatory factors.

STUDY MODELS

Biliary studies started over one hundred years ago. In 1902 Bayliss and Starling discovered the hormone secretin as an agent with stimulatory effects on pancreatic secretion and bile flow^[19]. About 25 years later secretin was reported as a cholagogue, i.e. an agent capable of stimulating the flow of bile into the duodenum^[20], as well as a choleric agent that could stimulate the production of bile in the liver^[21]. In spite of this, although the role of hepatocytes for bile generation was widely recognized quite early, the contribution of cholangiocytes to the production of bile with an adequate composition was accepted more recently. Knowledge of the cholangiocyte contribution was facilitated by the availability of experimental models and the development of both *in vivo* and *in vitro* sophisticated procedures.

In vivo models

The initial experimental studies on the bile flow and bile composition were carried out by using *in vivo* models of conscious or anesthetized mammals with biliary fistulas. In the case of total biliary fistulas, there was a major concern because of the interruption of the enterohepatic circulation and the subsequent depletion of the bile acid pool. However, this can be overcome by continuous intravenous or intraduodenal administration of exogenous bile acids at controlled rates. On the other hand, partial biliary fistula allows controlled interruption of the enterohepatic circulation, and a portion of bile collected can be returned to the stomach or duodenum, thereby replenishing the pool of natural bile acids^[10]. Fistula animals have also been useful to estimate canalicular bile flow by measuring the biliary clearance of selected inert solutes (mainly erythritol and mannitol). This procedure assumes that these solutes are sufficiently permeable to enter the canalicular bile by passive processes while being unable to cross the ductal epithelium^[22,23]. It appears that this might be the case in some animal species but not in all^[24]. Moreover, bile duct cannulation allows direct interventions in the biliary tract through retrograde intrabiliary injection^[25-28]; this procedure has been employed to obtain animal models of hepatitis^[29] and toxic-induced biliary disease^[30], as well as for experimental gene therapy^[31,32]. Retrograde intrabiliary injection has also been employed to assess the effects of toxic substances or inhibitors on the bicarbonate-rich choleresis upon stimulation. For instance, ursodeoxycholate-induced bicarbonate-rich choleresis has been shown to be sensitive to intrabiliary phenol^[33]. More recently, secretion of bicarbonate in secretin-stimulated rats has been found to be sensitive to intrabiliary administration of particular ion-transport blockers^[17].

In humans, *in vivo* assessment of biliary bicarbonate secretion has employed cumbersome maneuvers like nasobiliary drains in hepatic bile ducts^[34]. In a context of surgical interventions, invasive procedures similar to those employed in animals (for instance T-tube insertion into the common bile duct^[35,36] or percutaneous transhepatic cholangio-drainage^[37]) were also used. Recently, non-invasive assessment of biliary bicarbonate secretion

was developed by using positron emission tomography (PET)^[38]. This imaging technique allows evaluation of baseline and stimulated organ functions after intravenous injection of short half-live positron emitting isotopes^[39,40]. Thus, 2-3 min after bicarbonate labeled with carbon-11 (half live of 20.4 min) was given to healthy volunteers, label uptake was observed in the abdominal region corresponding to the liver parenchyma and hepatic hilum. Interestingly, administration of secretin increased bicarbonate uptake in the parenchymal region, this being followed by accumulation of the label in the perihilar area^[38]. Currently, the availability of micro-PET systems may also facilitate non-invasive *in vivo* studies of biliary bicarbonate secretion in small laboratory animals.

Animal models of bile ductal cell hyperplasia have also been developed, mainly in rodents, to study the pathophysiology of bile ducts^[41-43]. These models are closely associated with increased secretin-stimulated ductal secretion. The increased responsiveness seems to occur regardless of the procedure employed to obtain the hyperplasia of cholangiocytes: partial hepatectomy^[44], chronic feeding of bile acids^[45] or α -naphthylisothiocyanate (ANIT)^[46], chronic administration of phenobarbital plus CCl₄ to rats^[47] or just CCl₄ in mice^[48], or bile-duct ligation (BDL)^[41,49]. In contrast, a decreased secretin-stimulated ductal secretion can be observed in BDL rats with ductopenia following interruption of the cholinergic innervation by total vagotomy^[50], or selective damage of large (but not small) cholangiocytes by acute feeding with CCl₄^[51,52].

The development of genetically modified murine models has contributed to ascertain the role of selective genes, like those for the cystic fibrosis transmembrane conductance regulator *Cftr*^[53,54] and the P-glycoprotein P-glyc3/MDR2-3/ABCB4 *Mdr2/Abcb4*^[55], among others. In *Cftr*^{-/-} mice, for instance, induction of colitis has been shown to result in increased bile duct injury^[53]. In *Mdr2/Abcb4*^{-/-} mice, a multistep process of bile-duct damage leading to sclerosing cholangitis has been described^[55]. Spontaneous mutant animals can also serve as useful *in vivo* models. The PCK rat, a model of the autosomal recessive polycystic kidney and hepatic disease (PKHD), has been used for studies on possible trigger factors of biliary cystogenesis. This mutant was spontaneously developed in the rat strain Crj:CD/Sprague-Dawley because of a germ line mutation of the *Pkhd1* gene^[56,57]. The TR⁻ rat model is widely used for studying canalicular secretion of organic anions. This mutant lacks the functional canalicular isoform of the conjugate export pump MRP2/ABCC2 because of one-nucleotide deletion in the *Mrp2/Abcc2* gene^[58].

In vitro models

Isolated perfused liver preparations are useful experimental models to evaluate the liver effects of single factors in a manner independent of systemic or humoral effects^[59]. There are two modalities of these preparations, as the liver can be isolated and perfused *in situ* or *ex situ*, i.e. attached to or removed from the animal body. Livers can be isolated from many animal species (rat^[1,59], hamster^[60], guinea pig^[61], cat^[62], rabbit^[63], dog^[64], sheep^[65], calf^[66], pig^[67], and

monkey^[68]), the isolated perfused rat liver (IPRL) being the model most widely used^[69]. All these models allow repeated collection of both the perfusate and the bile, and permit easy exposure of the liver to different concentrations of test substances. Test substances may be given intravascularly through just the portal vein^[1,59-61,70], which provides flow to essentially all hepatocytes^[71], or through both the portal vein and the hepatic artery (i.e. isolated bivascularly perfused liver)^[72,73]. The latter bivascularly perfused model is particularly adequate to investigate ductal physiology because the predominant blood supply to the bile ducts is *via* the hepatic artery^[74,75]. For studies on ductal secretion, test substances and drugs may also be administered intrabiliary (as retrograde intrabiliary injection^[76]). Altogether, these *in vitro* preparations of isolated perfused liver had provided very valuable data on liver physiology and pathophysiology, the regulation of bile secretion included. However, extrapolation of that data to the *in vivo* situation should be cautious, as *in vivo* effects of local factors may be tightly influenced by systemic players such as humoral factors and innervation.

Membrane vesicle preparations derived from rat liver have been useful to distinguish transport across the cell membrane from intracellular events. They may be selectively enriched in basolateral or apical plasma membrane, or in intracellular membranes^[77]. The first identification of liver anion exchange activity was carried out in canalicular plasma membrane vesicles^[78].

While isolated couplets of hepatocytes was particularly useful as primary secretory units to study canalicular secretion^[79], the development in the last decade of the model of isolated bile duct units (IBDU), mainly from rat^[16,80,81], but also from mouse^[82] is making a great contribution to better study the regulation of ductal bile secretion^[83]. Both models permit micropuncture for electrophysiological studies as well as video microscopic optical planimetry to determine bile secretion^[16,81,84]. Isolated cholangiocytes^[85-89] are also widely employed for transport studies. The preparation of different size subpopulations of cholangiocytes and IBDU isolated from specific portions of the rat intrahepatic biliary tree has made it possible to define the functional heterogeneity of cells lining specific sized intrahepatic ducts^[2,90]. Furthermore, knockout and mutant animal models are being used to isolate cholangiocytes and bile duct units to carry out different *in vitro* studies^[91-93]. Moreover, studies have been performed in isolated cholangiocytes from patients with cystic fibrosis (i.e. patients with mutations in the *CFTR* gene)^[94,95]. Finally, a major advance is coming from the development of polarized primary cultures of intrahepatic cholangiocytes from both rat^[96,97] and humans^[98-101].

As the excretion of bicarbonate to bile involves a change in intracellular pH (pH_i) and a counterbalance through ion transporters in the responsible epithelial cells, several procedures and maneuvers have been developed to study pH_i regulation. The main strategy is based on the pH_i recovery towards its initial values after loading cells with acid or alkali. This type of experiment requires techniques for rapid and efficient monitoring of the pH_i. Although the most direct and accurate method seems to be

the insertion of double-barreled microelectrodes sensitive to [H⁺]_i into the cell, this has important limitations; tested cells should have large dimensions and a special ability to manipulate the electrodes. Thus a valuable alternative is microfluorimetry, a procedure based on the sensitivity of intracellular fluorescent dyes to pH. The indicator most widely employed is the membrane-permeable compound 2', 7'-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein-acetoxymethyl ester (BCECF-AM)^[102-104]. Uncharged BCECF-AM rapidly enters the cell, where it is enzymatically cleaved into its charged, membrane impermeant, fluorescent form BCECF. When bound to H⁺ ions, BCECF exhibits a shift in its emission fluorescence spectrum, and the ratio between the pH-sensitive emission at 495 nm wavelength, and the pH-insensitive (background) emission at the isosbestic point (440 nm) can be estimated. Once proper adjustments are set up (correcting for cellular autofluorescence, minimizing dye leakage, bleaching, or compartmentation, and preventing photodynamic damage to the cells), this methodology allows for continuous measurements of pH_i, with a very rapid response time. The technique can be applied to studies with a fluorimeter, flow cytometer, inverted epifluorescent microscope connected to a photon-counting photometer and a TV camera attachment plus a digital image-processing software^[105]. An example of successful use of this methodology is the measurement of the Na⁺-independent Cl⁻/HCO₃⁻ anion exchange (AE) activity in both cell clusters and single cells. After inducing intracellular alkalization by administration and further withdrawal of the cell-permeant weak acid propionate in Krebs-Ringer bicarbonate buffer (KRB), it is possible to estimate the anion exchange activity as the rate of spontaneous recovery of pH_i^[106]. Rates of pH_i recovery can be measured as $\delta\text{pH}_i/\delta t$ from the tangent to the experimental plot; transmembrane acid fluxes (or equivalent transmembrane base fluxes, i.e. J_{OH}) are usually calculated as $\beta_{\text{tot}} \times \delta\text{pH}_i/\delta t$, β_{tot} being the total intracellular buffering power in the presence of CO₂/HCO₃⁻, estimated as described^[107,108].

The collection of additional important methodologies which enable a continuous progress in bile secretion pathophysiology is still large. Thus, microcomputed tomography^[109], scanning and transmission electron microscopy^[89,110], immunoelectron microscopy^[111], and dual labeled immunogold^[112,113] are only a few among those techniques deserving a brief mention. Moreover, concerning molecular biological tools, the techniques of gene silencing through RNA-interference show great potential for clarifying the function of selective genes in bile duct cells. Currently, RNA-interference may be achieved with small interfering RNAs (siRNA) and through microRNAs (miRNA) and short/small hairpin RNA (shRNA)^[114]. siRNAs had been used in normal rat IBDU to examine the role of fibrocystin in ciliary morphology and biliary cystogenesis^[57] and that of aquaporin-1 (AQP1) in the transport of water by biliary epithelia^[115]. Also siRNA experiments had been carried out in cholangiocarcinoma cells to identify factors involved, for example, in the growth of these cells^[116] or their resistance to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)^[117]. Usual transfection protocols are not very efficient to internalize the siRNAs in cultured

cholangiocytes. Viral vectors that incorporate shRNA expression cassettes have thus been developed. Recently, a recombinant adenovirus with this design has been constructed and employed to efficiently infect normal rat cholangiocytes in culture and test the function of AE2/SLC4A2 anion exchanger in these cells^[17].

MECHANISMS INVOLVED

Bile formation is regarded as an osmotic water flow in response to active solute transport. Bile salts are secreted in the canaliculi through a specific export pump referred to as BSEP/SPGP/ABCB11 (from bile salt export pump, sister of P-glycoprotein, and ATP-binding cassette, subfamily B, member 11, respectively)^[118-120], allowing for the generation of the bile salt-dependent flow fraction. Remaining bile-salt independent flow fractions can be driven by supplementary solutes secreted at both the canaliculi (the so-called canalicular bile salt-independent flow), and the bile ducts (named as ductal bile salt-independent flow)^[1,2,10,14,121]. Estimates for the magnitude of each bile flow fraction in humans are shown in Table 1.

Canalicular bile-salt independent flow

In addition to bile acid secretion, the canalicular membranes of hepatocytes show active secretion of other organic and inorganic compounds, mainly glutathione^[13] and bicarbonate^[11,12,122], respectively. Glutathione can be secreted *via* the organic anion transporter MRP2/ABCC2^[123], while the efflux of bicarbonate occurs through a DIDS-sensitive Na⁺-independent Cl⁻/HCO₃⁻ exchange in association with other ion transport systems (Figure 1)^[78,124-127]. Both glutathione and bicarbonate seem to have an equivalent major input in canalicular bile flow generation, each driving up to 50% of the bile salt-independent fraction^[11]. For this to be accomplished, resultant osmotic forces need to be associated with aquaporin-mediated transcellular movement of water molecules from plasma to the bile canaliculi^[128]. In any event, the relative contribution of the canalicular bile salt-independent fraction to the whole canalicular bile flow may vary substantially between animal species (Table 2).

Canalicular bicarbonate excretion has been reported to be regulated by glucagon^[134,135], a hormonal oligopeptide secreted by the pancreatic α -cells. This hormone is encoded by the gene *GCG*, which belongs to the same multigene family as secretin (*SCT*), vasoactive intestinal peptide (*VIP*), and gastric inhibitory peptide (*GIP*) genes, among others. Following its synthesis in the pancreas, glucagon may reach the liver and stimulate the hepatocytes *via* its interaction with the glucagon receptor (Figure 2). Interestingly, this receptor and the receptors for secretin, VIP, GIP and other small peptidic hormones, are included in a superfamily of receptors characterized by a 7-transmembrane domain structure and by their coupling to adenylate cyclase (*ADCY*) *via* GTP-binding proteins (G proteins). Glucagon-glucagon receptor interaction in hepatocytes leads to increased intracellular levels of cAMP, PKA activation, stimulation of canalicular Cl⁻/HCO₃⁻ exchange activity^[126,134,135], and enhanced AQP8-mediated water permeability at the canalicular membrane^[128,136-138].

Table 1 Estimated bile flow rates in humans^[10]

Bile flow fraction	Flow rate
Canalicular bile acid-dependent flow	0.15-0.16 mL/min
Canalicular bile acid-independent flow	0.16-0.17 mL/min
Ductal secretion of bile flow	0.11 mL/min
Daily total bile flow	620 mL/d

Table 2 Estimations of canalicular bile-salt independent flow in different species

Species	Flow rate ^[122] (μ L/min/kg)	Percentage of the canalicular bile flow
Humans	2	30% ^[128,129]
Monkey	7	
Dog	5	20% ^[9]
Rat	70	> 60% ^[1,131,132]
Rabbit	60	> 50% ^[133]

Current data strongly suggests that these choleric effects of glucagon are microtubular-dependent and involve mobilization of intracellular vesicles^[126,128,136,139,140]. These effects of glucagon in hepatocytes resemble those of secretin in cholangiocytes (Figure 2). After their interaction with their specific G-protein-coupled receptors, both hormones appear to use a similar cAMP-dependent PKA pathway to co-redistribute cell-type specific intracellular vesicles with flux proteins towards the apical plasma membrane of the target cell. But some of the flux proteins involved in bile formation may differ between hepatocytes and cholangiocytes. While AQP8 is the involved water channel in hepatocytes^[128,136,137,140], it changes to AQP1 in cholangiocytes^[112,141,142]. Moreover, there are data suggesting that in hepatic cells in baseline situation, the water channel AQP8, the glutathione carrier MRP2/ABCC2 and the chloride bicarbonate exchanger AE2/SLC4A2^[138,140] are present in pericanalicular vesicles that might migrate to the canalicular membrane upon glucagon stimulation^[128]. These data, together with previous findings on AE2/SLC4A2 expression at the canaliculi and the pericanalicular area^[143,144], point towards the canalicular bicarbonate excretion occurring *via* an AE2/SLC4A2-mediated Cl⁻/HCO₃⁻ exchange^[127]. Although a number of observations suggest that this excretion of bicarbonate in exchange for chloride functions in connection with an apical chloride channel that maintains favorable Cl⁻ gradients^[78,124,127], the specific chloride channel(s) involved need yet to be identified (see Figures 1 and 2). While in cholangiocytes AE2/SLC4A2 colocalizes with CFTR^[112], this particular cAMP-responsive Cl⁻ channel is not expressed in hepatocytes. Therefore, it might be expected that a Cl⁻ channel other than CFTR^[145-148] be physically and/or functionally associated with AE2/SLC4A2 in hepatocytes. Moreover, previous findings have established an important link between bicarbonate excretion to bile and changes in pH_i. Canalicular Cl⁻/HCO₃⁻ exchange is a major hepatic acid loading mechanism; at resting pH_i and in the absence of any stimulation it shows low activity, but it is rapidly activated by intracellular alkalosis, whereas cAMP stimulation leads to intracellular acidification

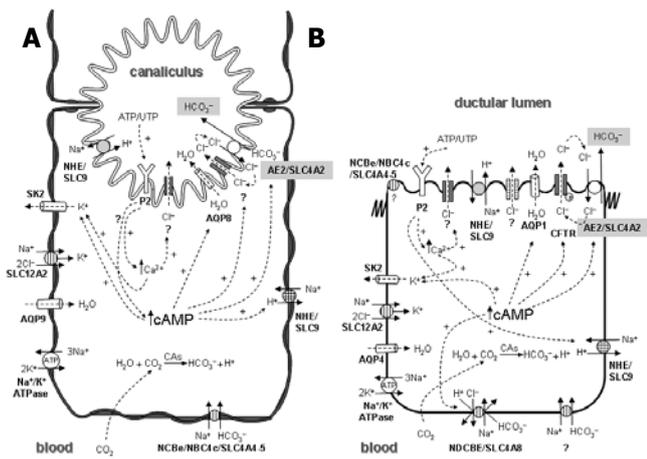


Figure 1 The major ion carriers implicated in pH_i regulation and secretion of bicarbonate to bile from liver cells. **A:** Hepatocytes; **B:** Cholangiocytes.

which requires appropriate counterbalance^[124,127]. Figure 1 summarizes several carriers putatively involved in these events in hepatocytes (as well as in cholangiocytes), including acid/base transporters and related proteins. A brief recall on bicarbonate loaders -and therefore acid extruders- highlights the role of electrogenic Na^+ - HCO_3^- cotransporters (NBCe)^[149-151] and the carbonic anhydrase/CA- CO_2 pathway^[152,153]. Intracellular load of bicarbonate may increase upon hydration of intracellular CO_2 by carbonic anhydrase(s)^[154] and subsequent H^+ efflux *via* Na^+ / H^+ exchange (NHE) (Figure 1). The involved exchanger is mainly the basolateral NHE1/SLC9A1^[155-157], but NHE4/SLC9A4^[158] and the canalicular NHE3/SLC9A3^[159] may also participate. The other relevant way of loading bicarbonate into hepatocytes, i.e. through electrogenic Na^+ - HCO_3^- cotransport, functions upon membrane depolarization following intracellular acidification^[160]. The specific proteins mediating this cotransport in hepatocytes remain to be defined. The only two members of the SLC4 family of bicarbonate transporters^[161] known to be electrogenic are NBCe1/SLC4A4 and NBCe2/NBC4/SLC4A5^[162]. NBCe1/SLC4A4 is widely expressed in most tissues, but its expression is negligible in the liver^[163,164], while mRNA expression for NBCe2/NBC4/SLC4A5 is high in this tissue^[165]. Of the known human NBCe2/NBC4/SLC4A5 variants, only the rat NBC4c ortholog can be detected in the rat liver by RT-PCR^[164]. Moreover, NBC4c immunoreactivity had been observed at the basolateral membrane of rat hepatocytes^[164]. Altogether these findings suggest that the basolateral NBC4c variant might be a relevant bicarbonate loader that enables hepatocytes for canalicular secretion of bicarbonate through the apical AE2/SLC4A2 anion exchanger.

Ductal bile

As previously mentioned, primary canalicular bile undergoes a process of fluidization and alkalinization along the biliary tract that is influenced by several factors including hormones (mainly secretin in most species^[2,14-17]), innervation/neuropeptides^[2,50,166,167], and biliary constituents^[2,5,6,17,18]. This process results in net ductal secretion

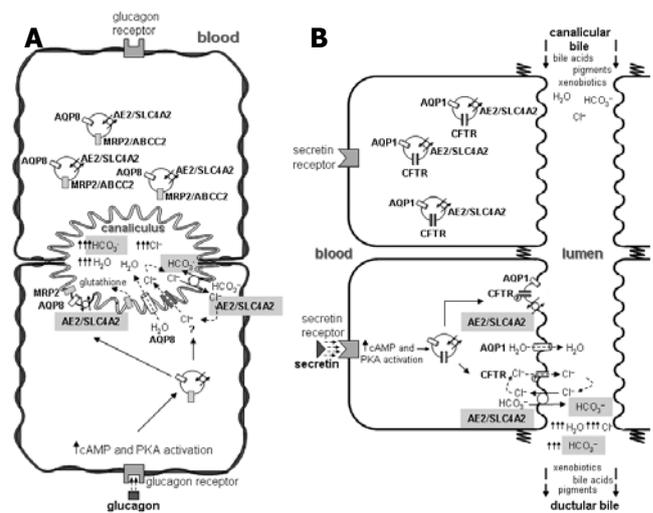


Figure 2 Hormonal stimulation of bicarbonate excretion from hepatocytes and cholangiocytes. **A:** In hepatocytes, glucagon may induce an exocytic trafficking of vesicles with the anion exchanger AE2/SLC4A2 and the water channel AQP8, which can result in canalicular bicarbonate-rich hydrocholeresis. MRP2/ABCC2, while being a carrier for organic anions like glutathione rather than an inorganic ion transporter, is hereby depicted because of its possible colocalization with the other two flux proteins; **B:** In cholangiocytes, secretin induces trafficking of vesicles with the chloride channel CFTR, the anion exchanger AE2/SLC4A2, and the water channel AQP1, and further exocytosis to the luminal membrane of these cells, which results in ductal bicarbonate-rich hydrocholeresis.

of a bicarbonate-rich watery fluid. The magnitude of the ductal contribution varies between species, representing 30% of basal bile flow in humans and 10% in rats^[6]. Cholangiocytes are provided with specific transport systems that participate in bile modifications^[2,168]. They are able to take up bile salts *via* an apical Na^+ -dependent transporter (SLC10A2, formerly ASBT/ISBT and occasionally ABAT)^[45,169-172] and release them through a basolateral truncated isoform of the same carrier^[173]. Indeed, transcellular transport through these carriers is specially important under cholestatic situations. In any case under normal conditions, biliary transport of bicarbonate appears as a relevant function of the bile duct epithelium. It is accomplished by specific acid/base carriers and related transporters that enable cholangiocytes to regulate their pH_i ^[105,174] (Figure 1).

Cholangiocyte acid extruders

The vectorial transport of HCO_3^- from cholangiocytes to duct lumen starts with the accumulation of HCO_3^- in the cells *via* mechanisms which vary between animal species. In rat cholangiocytes, for instance, HCO_3^- loading is mediated by transport systems similar to those in hepatocytes, i.e. by electrogenic Na^+ - HCO_3^- cotransport activity^[168,174-176] and the carbonic anhydrase/CA- CO_2 pathway coupled to subsequent carrier-mediated H^+ extrusion^[155,154]. Carbonic anhydrases (CAs) catalyze the hydration of carbon dioxide, $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+$ (reviewed in ref. 153). Thus far, several CA isoenzymes have been identified which differ in organ distribution, subcellular location, and function (cf. Table I in ref. 153). Compared with other secretory organs, the mammalian liver contains relatively low levels of total CA activity. Thus, the cytoplasmic CA-II (CA2)

is mainly expressed in bile duct cells (but it may also be found in hepatocytes), and appears to be involved in the production of HCO_3^- for its further secretion to bile^[153,177]. Also, the biliary epithelial cells express the membrane associated CA-IV and CA-IX, which on the basis of their location might be involved in acidification and concentration of the bile, even though the exact mechanisms have not been described^[153].

Among electrogenic $\text{Na}^+/\text{HCO}_3^-$ cotransporters^[161,162] only the NBC4c variant of NBCe2/NBC4/SLC4A5 was found to be expressed in the rat liver, being immunolocalized to both hepatocytes and cholangiocytes^[164]. Interestingly, NBC4c immunoreactivity was basolateral in hepatocytes but apical in cholangiocytes, suggesting a potential role for this cotransporter in the luminal fluid secretion and/or absorption^[164]. In humans cholangiocytes, however, $\text{Na}^+/\text{HCO}_3^-$ cotransport is not active in the physiological range of pH_i , being active only at very low pH_i , and bicarbonate influx occurs mainly through electroneutral Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ anion exchange^[176,178]. Thus far, NDCBE/SLC4A8 is the only Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ exchanger cloned in humans^[179]. Although Northern blot analysis could not detect its messenger RNA in the whole liver^[179], this cannot rule out that NDCBE/SLC4A8 be expressed in cholangiocytes (which account for just 5% of the liver cell population).

In both rat and human cholangiocytes, H^+ extrusion takes place essentially through NHE activity (Figure 1) -while in pig cholangiocytes H^+ extrusion is mediated by a cAMP-activated H^+ -ATPase^[180]. Thus far, several members of the NHE/SLC9 family have been described in rat cholangiocytes. They include NHE1/SLC9A1, restricted to the basolateral membrane and highly sensitive to amiloride, and the amiloride-insensitive isoform NHE2/SLC9A2, which is likely to be active on the side facing the lumen^[106]. Moreover, NHE3/SLC9A3 has been immunolocalized to the apical membrane of rat cholangiocytes, where it may play an important role in fluid absorption from the bile duct lumen^[159]. Also NHE4/SLC9A4 -an isoform seemingly activated by hypertonicity and with K^+/H^+ exchange activity^[181], was identified in whole liver extracts by Western blot^[158]. Some findings in the stomach of *Nhe4/Slc9a4*^{-/-} mice had led to speculations on a possible functional coupling of NHE4/SLC9A4 with the AE2/SLC4A2 anion exchanger in parietal cells^[182]. In any case, the specific cellular expression and function of this NHE isoform in the liver remains to be determined.

Cholangiocytes acid loaders

As previously reported in hepatocytes, the main acid loader mechanism in bile duct cells is the apical Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchange^[17,106,174,176]. Such an AE activity might secrete HCO_3^- into the lumen which is exchanged for Cl^- influx. This exchange is electroneutral, being facilitated by the outside to inside transmembrane gradient of Cl^- at relatively high intracellular concentration of HCO_3^- , specially upon secretin stimulation^[16,81,106,175,183] (Figure 2). Actually, several bicarbonate transporters have been described as exerting Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchange activity. This is the case for the SLC4 anion exchangers (AE1/SLC4A1, AE2/SLC4A2 and AE3/SLC4A3)^[161,184] as

well as several members of the SLC26 gene family of multifunctional anion exchangers (DRA/CLD/SLC26A3, PDS/DFNB4/SLC26A4, and SLC26A6^[185] and more controversially SL26A7^[186,187]). But none of these carriers except AE2/SLC4A2 had been described to occur in the liver. Moreover, AE2/SLC4A2 was localized not only to the canalicular but also to the luminal membrane of bile duct cells^[143]. Recent experiments of RNA interference with recombinant adenovirus expressing shRNA have shown that AE2/SL4A2 is indeed the main effector of both basal and stimulated Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchange in rat cholangiocytes^[17].

Other ion-loaders/extruders

Besides acid/base transporters cholangiocytes possess other ion carriers like those for Cl^- , Na^+ , and K^+ , which greatly contribute to pH_i regulation and bicarbonate secretion (Figure 1). Thus, the cAMP-responsive Cl^- channel CFTR had been localized at the apical side, where it plays a role in biliary excretion of HCO_3^- ^[188,189]. Although HCO_3^- permeability through activated CFTR has been shown in several cell systems^[190-194], its main contribution to biliary bicarbonate secretion appears to occur through a coordinated action with AE2/SL4A2^[17,175,195]. In addition to CFTR, cholangiocytes possess a dense population of Ca^{2+} -activated Cl^- channels. These channels are responsive to interaction of the purinergic-2 (P2) receptors with nucleotides (mainly ATP or UTP)^[196-199], and they appear to be related to the Ca^{2+} -calmodulin-dependent protein kinase II^[188,200,201]. Resultant Ca^{2+} -stimulated Cl^- efflux might be up to 2-fold greater than that mediated by cAMP^[202]. Additional high conductance anion channels which are insensitive to both Ca^{2+} and cAMP were identified in rat bile duct cells^[203], but their specific role remains to be defined. The efflux of Cl^- from cholangiocytes to the ductular lumen is counterbalanced not only *via* apical $\text{Cl}^-/\text{HCO}_3^-$ exchange but also through other Cl^- uptake systems, mainly the basolateral $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter NKCC1/SLC12A2^[196,204]. In addition to maintain high intracellular Cl^- concentration facilitating apical Cl^- excretion, this forskolin-stimulable cotransporter has been reported to participate in cell volume homeostasis^[205] and cell proliferation^[204,206,207]. Because the NKCC1/SLC12A2-mediated influx of Cl^- occurs together with the entry of Na^+ and K^+ , cholangiocytes possess other systems that sustain the gradients of these cations and the membrane potential difference. Thus a basolateral sodium pump (the $\text{Na}^+/\text{K}^+/\text{ATPase}$)^[208,209] extrudes Na^+ and uptakes K^+ with a 3:2 stoichiometry^[210]. Accumulation of K^+ within cholangiocytes is prevented by its exit through K^+ channels. Intracellular cAMP and/or Ca^{2+} concentration may activate basolateral K^+ conductance that hyperpolarizes the cell^[211]. The small conductance K^+ channel SK2/KCNN2 has been identified in rat and human hepatocytes^[212] and in rat and human biliary epithelia^[202,212], the activity of which is stimulated by small increases in intracellular Ca^{2+} in an apamin-sensitive manner^[213]. Although SK2/KCNN2 immunoreactivity has been localized in cholangiocytes at both apical and basolateral membranes, functional studies in polarized preparations have demonstrated a significantly greater basolateral Ca^{2+} -stimulated K^+ conductance^[202].

Because the nonselective K^+ channel blocker Ba^{2+} is able to cause a greater degree of inhibition than apamin^[202], apamin-insensitive channels not yet identified may also be involved in the conductance of K^+ .

In a context of cAMP- and/or Ca^{2+} -stimulated Cl^- channels, cell hyperpolarization due to K^+ conductance facilitates the transcellular Cl^- movement into the lumen. Recycling of K^+ can be held up through the basolateral $Na^+-K^+-2Cl^-$ cotransport and the Na^+/K^+ -ATPase. All these ionic fluxes across cholangiocyte membranes may ultimately lead to biliary excretion of bicarbonate *via* its exchange with luminal chloride, which is facilitated by the outside to inside transmembrane gradient of chloride at relatively high intracellular concentration of bicarbonate^[16, 81,106,175,183]. The apical fluxes of anions result in increased osmotic forces in the bile duct lumen which in the presence of aquaporins contribute to the generation of ductal bile flow. This view has been strongly supported by the finding that AE2/SLC4A2 and CFTR both colocalize with AQP1 in cholangiocyte intracellular vesicles which co-redistribute to the apical cholangiocyte membrane upon both cAMP and secretin stimulations^[112].

Water transporters or aquaporins

Aquaporins are water channels that mediate a bidirectional passive movement of water molecules across epithelial cells in response to osmotic gradients established by ions and solutes. Thus far, rodent cholangiocytes have been described to possess two types of aquaporins. Thus, in addition to the AQP1 locating in the intracellular vesicles which may traffic to the apical membrane upon agonist stimulation^[112], there occurs another water channel at the basolateral membrane of cholangiocytes named AQP4, which is not sensitive to secretin^[214,215] (Figures 1 and 2). However, there can be differences between species. For instance, immunohistochemical analysis in liver pig has shown the presence of AQP1 and AQP9 in cholangiocytes, while AQP3, AQP4, AQP7, and AQP8 were absent in these cells^[216].

REGULATORY FACTORS

The bile duct epithelium is constantly regulated by the action of multiples factors that contribute to the formation of bile flow with an adequate final composition. Among these factors secretin is a relevant hormone peptide which may induce bicarbonate-rich hydrocholerisis in many animal species. Thus, secretin regulation of ductal bile flow and the concurrence of other factors are briefly summarized as follows:

Relevance of secretin stimulation

In 1902, Bayliss and Starling^[19] described an active agent originating from the intestinal mucosa, which they referred to as secretin because of its capacity to stimulate pancreatic secretion in the dog. They also used the word "hormone" to designate this sort of compound that can be produced in an organ and carried through the circulation to exert its effect on another organ. Some years passed before the choleric effect of secretin in the liver was reported in several animal species and humans^[217-219]. Secretin was

found to stimulate bile flow together with a decrease in the concentration of bile salts^[217-219]. In the late 1960s the role of bile ducts in secretin-induced hydrocholerisis was postulated because of the observation that this secretin effect was associated with reciprocal changes in the biliary concentration of bicarbonate and chloride anions^[35].

Meanwhile secretin was purified from most species, first in 1962 from pig^[220,221] and then from humans, dog, goat, guinea pig, rabbit, rat, mouse and chicken. Secretin has only 27 amino acids, which allowed for its chemical synthesis as early as 1968^[222]. There is close homology between mammalian secretins, but also between the regulatory peptides (more than 10) currently grouped in the secretin/glucagon/ VIP superfamily.

Secretin is produced in many organs but mainly in the mucosa of upper small intestine (duodenum and jejunum)^[223,224]. Its release to blood occurs mainly in the postprandial period, being stimulated by gastric acid delivered into duodenal lumen^[225], as well as by pancreatic and intestinal secretions^[226,227]. Secretin release appears to be mediated by a luminal secretin-releasing peptide contained in these gastrointestinal juices^[228-230]. In addition to its mentioned biliary and pancreatic effects, secretin may function as feedback inhibitor of gastrin (GAST) release and gastric acid secretion^[231], and may also regulate gastric motility^[232]. The physiological activities of secretin are subjected to hormone-hormone and neuro-hormonal interactions. Inhibition of gastric acid secretion by secretin is thus mediated by somatostatin (SST) and prostaglandins^[233,234], and secretin inhibition of gastric motility involves a vagal afferent pathway^[235,236].

Secretin exerts its physiological actions *via* interaction with the N-terminal extracellular tail of its specific glycoprotein receptor SCTR^[237-239]. Like the glucagon receptor and other members of the same receptor superfamily, SCTR is coupled to adenylate cyclase/ADCY through an oligomeric GTP-binding protein. In the liver SCTR is exclusively expressed at the basolateral membrane of cholangiocytes^[240,241]. As previously noted, the action of secretin in cholangiocytes runs parallel with the choleric effect of glucagon in hepatocytes (Figure 2). Thus, both glucagon and secretin may stimulate the bile salt-independent bile flow, but each at a different level, i.e. canalicular for the former and ductular for the latter(cf. ref. 242).

Secretin-SCTR interaction in cholangiocytes results in increased intracellular levels of cAMP^[15,16,243]. Further PKA activation^[175,183] can induce microtubule-dependent co-redistribution of the intracellular vesicles with AE2-CFTR-AQP1 flux carriers to the apical membrane^[112]. Additionally, the CFTR is phosphorylated and activated^[244], thus resulting in Cl^- efflux to the ductular lumen. In consequence, Cl^- secreted by CFTR can be exchanged with HCO_3^- through AE2/SLC4A2. This mechanistic model has been consistently confirmed *in vitro* using rat IBDU^[116,175] and cholangiocytes^[174]. For *in vivo* studies, the most experiments with rats have used some of the aforementioned models with bile duct proliferation^[41,43,46, 112,245,246], since normal rats appear to respond very poorly to secretin (likewise rabbit, but in contrast with guinea pig among rodent species)^[15,41,46,47,247]. Indeed the expression of

SCTR is increased in BDL rat cholangiocytes^[245,246] but the receptor is also expressed in normal rat cholangiocytes^[240]. In the model of IPRL^[248], intra-arterial secretin increased biliary concentration of bicarbonate, but had no effect on the net bile flow. Because the effect of secretin was blocked only by the CFTR inhibitor NPPB and not by the anion exchanger inhibitor DIDS (both administered intra-arterially), it was proposed that Cl⁻/HCO₃⁻ exchange would have no role in the ductal secretion of bicarbonate in the normal rat^[248]. However, recent experiments indicate that secretin does increase bile flow and biliary Cl⁻ and HCO₃⁻ excretions in the normal rat, but when they maintain the bile acid pool *via* continuous infusion with taurocholate^[17]. Moreover, these effects of secretin were distinctively blocked by the inhibitors given by intrabiliary retrograde injection. While secretin effects were all blocked by intrabiliary NPPB, DIDS only inhibited secretin-induced increases in bile flow and bicarbonate excretion but not the increased chloride excretion^[17]. These findings provide evidence for the role of biliary Cl⁻/HCO₃⁻ exchange in secretin-induced bicarbonate-rich choleresis in the normal rat model.

The role of the bile acid pool

In line with former findings in earlier experiments with dogs^[14,249], the aforementioned observation that secretin also has effects on the normal rat when infused continuously with taurocholate^[17] confirms the notion that bile acids are relevant for secretin actions. In a previous study in normal rats secretin and taurocholate were tested and no secretin effects were observed^[41]. But in that study, taurocholate infusion was interrupted before secretin administration^[41]. It is already known that bile acids can enter into cholangiocytes through the carrier ASBT^[45,169-172] and exert their effects on these cells (reviewed in refs. 2 and 250). For instance, it has been recently reported, activation of CFTR by ASBT-mediated bile salt absorption, which is seemingly independent from cAMP or cGMP signaling^[251].

The bile salt-dependent canalicular flow is related to the osmotic activity of bile acids, but some bile acids such as ursodeoxycholic acid (UDCA), 23-nor-UDCA, and 23-nor-chenodeoxycholate^[252-254], have a higher choleric effect than can be accounted for by their secretion into bile (the so-called hypercholeric effect). This hypercholeric effect is associated with a marked stimulation of bicarbonate secretion into bile. A classic hypothesis referred to as "cholehepatic shunt pathway"^[255] claimed that the hypercholeric effect may involve intraductal protonation of unconjugated bile salts which results in the formation of bicarbonate anions derived from hydrated CO₂ (i.e. H₂CO₃ or H⁺ plus HCO₃⁻). Passive diffusion of uncharged bile acids through cholangiocytes to the periductular vessels and further uptake in hepatocytes could be followed by their canalicular resecretion as unconjugated bile salts (reviewed in ref. 250). After the identification of apical and basolateral bile acid carriers in cholangiocytes (ASBT and tASBT, respectively^[45,169-173]), the cholehepatic shunt hypothesis has received a boost, being updated for the conjugated bile salts^[250]. ASBT activity acutely increases upon secretin stimulation^[172], which may accentuate the cholehepatic bile acid shunting in the

postprandial period.

Neurovegetative liver innervation

The liver is directly regulated by the neurovegetative innervation. Indeed, the release of the neurotransmitter acetylcholine from the intrahepatic parasympathetic terminals induces, *via* selectively interaction with M₃ Ach receptors on cholangiocytes, an increase in both secretin-stimulated cholangiocyte cAMP synthesis and Cl⁻/HCO₃⁻ exchanger activity by Ca²⁺-calcineurin-mediated PKC-independent modulation of adenylate cyclase/ADCY^[255,256]. In fact, infusion of acetylcholine in the IPRL model was found to potentiate the effect of secretin on biliary HCO₃⁻ excretion^[248]. Furthermore, interruption of parasympathetic innervation in BDL rats by vagotomy has been reported to inhibit secretin-stimulated ductal secretion, as well as to decrease cholangiocyte intracellular cAMP levels^[50].

The intrahepatic biliary epithelium also receives dopaminergic innervation^[257,258], but in contrast to the cholinergic system, the dopaminergic system inhibits secretin-induced choleresis. Although both systems exert their functions through increased intracellular ions (1, 4, 5) P₃ and Ca²⁺, the cholinergic system acts *via* calmodulin and calcineurin but without recruitment of PKC^[256], whereas the D₂ dopaminergic system inhibits secretin-stimulated ductal secretion through increased expression and activation of PKC-γ^[259].

Moreover, cholangiocyte secretion can also be regulated by the action of the adrenergic system^[260,261]. The α₂-adrenergic receptor agonist UK-14304 has been reported to inhibit cholangiocarcinoma growth through time course-dependent modulation of Raf-1 and B-Raf activities^[260], and the α₁-adrenergic agonist phenylephrine can potentiate secretin-stimulated ductal secretion through the amplification of the ADCY system *via* a Ca²⁺- and PKC-dependent mechanism^[261].

Hormones

In addition to secretin, there are gastrointestinal hormones and neuropeptides such as bombesin/gastrin releasing peptide (BN/GRP), VIP, endothelin-1 (ET1/EDN1), somatostatin/SST, and gastrin/GAST, which may also modulate the ductular bile salt-independent flow (reviewed in ref. 2). Some of these factors operate through a secretin-independent mechanism, while others influence the release of secretin or interact with the secretin signaling in cholangiocytes, depending very much on the animal species. For instance, the neuropeptide bombesin/BN/GRP can act either by increasing the secretin release in dogs^[262,263], or inducing ductal secretion with activated Cl⁻/HCO₃⁻ exchange *via* secretin-independent mechanisms in isolated rat cholangiocytes^[2,264-266]. The effect of VIP on cholangiocytes depends also on the animal species^[2,267-271]. While VIP appears to increase secretin-stimulated bile flow and bicarbonate excretion in humans^[267,268], studies in rat IBDU show that VIP can stimulate basal fluid and bicarbonate secretion through a cAMP-independent pathway^[2,269].

The cyclic tetradecapeptide somatostatin/SST is able to inhibit basal and secretin-stimulated bicarbonate-rich choleresis *via* its interaction with the SSTR2 receptor

subtype^[2,272]. In rat, inhibition of secretin-stimulated ductal secretion by SST is associated with decreased expression of the secretin receptor SCTR in cholangiocytes and reduced secretin-stimulated cAMP levels^[49,272]. In mice SST has also been shown to stimulate ductal fluid absorption, a process involving intracellular cGMP synthesis and inhibition of secretin-stimulated cAMP synthesis^[273]. Moreover, in dogs, SST has been shown to diminish the acid-induced release of secretin from the duodenal mucosa^[262]. Similarly SST, the gastrointestinal hormone gastrin/GAST may also modulate cholangiocyte secretion^[2]. In BDL rats, GAST has been reported to inhibit secretin-stimulated ductal secretion by reducing both SCTR expression and secretin-induced cAMP levels^[274].

Other peptide hormones like insulin and insulin-like growth factor 1 (IGF1) can also modulate the biliary epithelium. Insulin was reported to inhibit secretin-induced secretion in BDL rats through activation of PKC and inhibition of secretin-stimulated cAMP and PKA activity^[275]. On the other hand, studies using a liver cell line (though from hepatoma rather than cholangiocyte type), showed that insulin may stimulate membrane turnover with increased exocytosis/endocytosis of vesicles containing ion channels^[276]. In rats IGF1 was found to stimulate choleresis^[277] as well as cholangiocyte proliferation^[278]. IGF1 can be synthesized and released from cholangiocytes under the control of the growth hormone (GH)^[278]. Biliary IGF1 may in turn interact with its receptor (IGF-R) located at the apical pole of cholangiocytes^[278]. Expression of both IGF1-R and IGF1 is markedly enhanced in cholangiocytes following bile duct ligation^[278].

Also steroidal hormones like corticosteroids and estrogens have effects on the biliary epithelium. Corticosteroids are choleric and increase biliary bicarbonate excretion^[277,279]. However, estrogen-induced cholestasis results in diminished biliary bicarbonate excretion^[280]. Reduced bicarbonate excretion might be caused by a reflux of biliary bicarbonate *via* leaky tight junctions as it is not associated with impaired activity of the Cl⁻/HCO₃⁻ exchanger^[280].

Purinergic stimulation

Both hepatocytes and cholangiocytes are able to release ATP and UTP, which leads to increased biliary concentration of nucleotides and nucleosides (the latter being a result of nucleotide dephosphorylation by membrane-associated nucleotidases)^[281-283]. Stimulation of the different subtypes of purinergic receptors (PR) at the luminal membrane of cholangiocytes by either extracellular nucleosides (P1 family receptors) or extracellular nucleotides (P2 family receptors) may control cholangiocyte secretion and ion channel activities^[281,283,286]. Most subtypes of purinergic receptors are G protein-coupled receptors except the P2X subtypes which are ligand-gated channels^[281,283]. P2 activation stimulates cholangiocyte biliary efflux of K⁺, HCO₃⁻, and Cl⁻, and reabsorption of Na⁺^[281,283,286]. Whereas Cl⁻ efflux seems to be mediated by a calcium-stimulated Cl⁻ channel^[284], HCO₃⁻ is secreted from cholangiocytes *via* an AE2/SLC4A2 mediated Cl⁻/HCO₃⁻ exchange^[17,105,174,284,287]. Of notice, the P2Y₁₁ subtype receptor has been reported to mediate secretion *via* cAMP in pancreatic duct epithelial cells^[288],

which suggests that a similar mechanism may also occur in cholangiocytes (as well as in hepatocytes). Finally, P2 receptors may stimulate the basolateral NHE1/SLC9A1 activity in cholangiocytes^[287].

Cytokines and related factors

Some cytokines such as IL5 and the combination of the proinflammatory cytokines IL6, IFN- γ , IL1, and TNF- α can inhibit secretin-induced choleresis^[289,290]. Moreover, proinflammatory cytokines can impair the barrier function of biliary epithelia^[290], and stimulate the biliary epithelium to generate NO, *via* induction of inducible nitric oxide synthase 2A (NOS2A/INOS). Resultant reactive nitrogen oxide species (RNOS) may cause ductular cholestasis through inhibition of both the soluble adenylate cyclase (SAC) and the cAMP-dependent HCO₃⁻ and Cl⁻ secretory mechanisms^[195]. Such a pathogenetic sequence may contribute to ductal cholestasis in inflammatory cholangiopathies^[195].

In conclusion, biliary secretion of bicarbonate is an important contributor to the generation of the bile-salt independent flow. Bicarbonate is secreted from both hepatocytes and cholangiocytes through parallel mechanisms which involve chloride efflux through activation of Cl⁻ channels, and further bicarbonate excretion *via* AE2/SLC4A2-mediated Cl⁻/HCO₃⁻ exchange. Glucagon and secretin are two relevant hormones which act very similarly in hepatocytes and cholangiocytes, respectively. These hormones interact with their specific receptor, resulting in increased intracellular cAMP levels and activation of cAMP-dependent Cl⁻ and HCO₃⁻ secretory mechanisms. Both hepatocytes and cholangiocytes seem to have cAMP-responsive intracellular vesicles in which AE2/SLC4A2 may colocalize with cell specific Cl⁻ channels (CFTR in cholangiocytes and thus far undetermined in hepatocytes) and aquaporins (AQP1 in cholangiocytes and AQP8 in hepatocytes). cAMP-induced coordinated trafficking of these vesicles to either canalicular or cholangiocyte luminal membranes and further exocytosis results in increased osmotic forces and passive movement of water with net bicarbonate-rich hydrocholeresis.

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