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TOPIC HIGHLIGHT

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Physiology of bile secretion

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Abstract

The formation of bile depends on the structural and functional integrity of the bile-secretory apparatus and its impairment, in different situations, results in the syndrome of cholestasis. The structural bases that permit bile secretion as well as various aspects related with its composition and flow rate in physiological conditions will first be reviewed. Canalicular bile is produced by polarized hepatocytes that hold transporters in their basolateral (sinusoidal) and apical (canalicular) plasma membrane. This review summarizes recent data on the molecular determinants of this primary bile formation. The major function of the biliary tree is modification of canalicular bile by secretory and reabsorptive processes in bile-duct epithelial cells (cholangiocytes) as bile passes through bile ducts. The mechanisms of fluid and solute transport in cholangiocytes will also be discussed. In contrast to hepatocytes where secretion is constant and poorly controlled, cholangiocyte secretion is regulated by hormones and nerves. A short section dedicated to these regulatory mechanisms of bile secretion has been included. The aim of this revision was to set the bases for other reviews in this series that will be devoted to specific issues related with biliary physiology and pathology.

Key words: Hepatocytes; Cholangiocytes; Bile flow; Bile acid; Transport

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INTRODUCTION

In 1924 Cramer and Ludford[1] published a paper in which they indicated: "liver cells present an unusual problem of secretory cellular activity. The cells of all other secreting glands are functionally unipolar, but hepatocytes are bipolar". Furthermore they wrote that "all the known facts compel a return to the old conception of Claude Bernard that glycogenic function represents an internal secretion of the liver". It is clear that bile secretion is the external one. Finally, regarding this functional bipolarity, those authors reported "it is difficult to form a mental conception of the cellular mechanism which enables one cell to pass two different specific secretions in different directions"; towards sinusoids or towards canaliculi. In that paper they offered morphological evidence that the Golgi apparatus is involved in secretion by hepatocytes in both directions.

Since then our understanding of bile secretion has evolved, and differerent attempts have been made to resolve diverging conceptual theories. In the fifties we learned that bile flow is not the result of a hydrostatic perfusion[2] but the result of osmotic forces[3]. In the sixties we began to distinguish between canalicular and ductular bile[4,5] and showed that bile flow is related to the amount of bile salts secreted to the canaliculi[6]. Shortly after, it was shown that the canalicular bile may be either dependent or independent of bile salts[7,8].

The use of different methods and techniques, such as chronic bile fistula[9], isolated and perfused livers[10], couplets of hepatocytes[11], isolated bile duct units[12], separation of membranes from basolateral and apical domains[13] and several other experimental approaches have helped us to advance our knowledge of the different mechanism involved in the formation of bile flow.

Over the last two decades molecular biology

techniques have allowed the cloning of different proteins expressed in cholangiocytes[14] and/or hepatocytes; such proteins may be involved in the transport of endogenous or exogenous organic anions and cations[15]. The overall role of these transporters depends on whether they are targeted from the Golgi apparatus to the apical or basolateral poles of the epithelial liver cells[16,17]. This may help to resolve the conceptual problem of bipolarity, pointed out by Cramer and Ludford. The discovery of nuclear receptors and plasma membrane receptors for bile acids (BAs)[18,19] has opened a new field of investigation regarding the role of these molecules in the control of secretory and metabolic mechanisms[20,21] that somehow links different aspects of endogenous and exogenous secretions to metabolic functions in parenchymal liver cells. This issue will be the matter of a separate review in this series.

In this introductory review, a brief revision is made of the structural bases that permit bile secretion as well as several aspects related to its composition and flow rate under certain physiological conditions. Knowledge of the role in bile flow generation of cholangiocytes has increased exponentially over the last few years[22,23] and is therefore reviewed here. Finally, a short section dedicated to the regulatory mechanisms of bile secretion is included. This should set the bases for other reviews in this series that will be devoted to specific issues related to biliary physiology and pathology.

STRUCTURAL BASES

The biliary apparatus is a convergent system of canals that begins in the canaliculi, followed by the bile ducts, and ending with the common bile duct (coledochus). Bile secretion depends on the function of membrane transport systems in hepatocytes and cholangiocytes and on the structural and functional integrity of the biliary tree. The hepatocytes, constituting the most abundant liver cell population (65%), generate the so-called primary bile in their canaliculi[4]. Biliary canaliculi are blind tubular structures, with a very high surface/volume ratio that favors-by means of osmotic gradients-the formation of bile flow[5]. Cholangiocytes, which constitute 3%-5% of the liver cells[24], modify the canalicular bile by secretory and reabsorptive processes as bile passes through the bile ducts[22], and they are responsible for approximately 30% of bile volume[25]. In contrast to hepatocytes, where secretion is constant and poorly controlled[26], cholangiocytes secretion is broadly regulated[22,27].

Hepatocytes exhibit structural and functional polarity, three different zones being distinguished in their membranes. The sinusoidal membrane, which faces Disse's space, covers 37% of the total surface of the hepatocyte. There is no lamina basal between hepatocytes and endothelial cells, which are fenestrated and show abundant vacuole of endocytosis and exocytosis, accounting for the intense exchange of substances between blood and hepatocytes[28]. In the lateral membrane (50% of the total surface) there are specialized structures that allow adhesion (desmosomes and tight-junctions) and comunication (gap-junctions) between adjacent hepatocytes. The tight-junctions determine the exchange of fluids and electrolytes between Disse's space and the canalicular space through the paracelular pathway[28]. Together, the sinusoidal and lateral membranes cover the basolateral surface (basolateral membrane). The canaliculi are tiny ducts delimited by the canalicular or apical membrane membrane of two adjacent hepatocytes[28] and they represent a small fraction of the total hepatocyte surface area.

Table 1 Membrane transporters in hepatocytes

Abbreviation	Function
Basolateral membrane (sinusoidal)	
NTCP/SLC10A1[1]	Takes up BAs
OATP/SLC21A[1]	Takes up BAs and OA^- and exports BAs, GSH, HCO_3^-
MRP3/ABCC3	Export OA^- conjugates, GSH
MRP4/ABCC4	Export BAs, GSH
OSTa /OSTb	Organic solute transporter: Exports BAs
NBC4c/SLC4A5[1]	Na^+-HCO_3^- symporter, acid extruder
NHE1/SLC9A1[1]	Na^+/H^+ exchanger, acid extruder
SK2	Potassium channel, potassium efflux
SLC12A2	Na^+-K^+-Cl^- symporter: Sodium, potassium, chloride uptake
Apical membrane (canalicular)	
BSEP/ABCB11[1], formerly SPGE	Bile salt export pump
MRP2/ABCC2[1], formerly cMOAT	Export non BAs OA^-, GSH
MDR1	Efflux of lipofilic cations
MDR3/ABCB4	Phospholipid flipase
ABCG5/ABCG8	Export sterols
AE2/SLC4A2[1]	Cl^-/HCO_3^- anion exchanger: Acid loader
Cl- channel[1]	Export chloride
AQP8[1]	Water channel
NHE3/SLC9A3[1]	Na^+/H^+ exchanger: Acid extruder

[1]Transporters relevant to bile flow under physiological basal conditions.

Currently, the molecular biology and genetic characteristics of many transporters of the basolateral and apical membranes are known (Table 1). Such transporters take part in the transfer of substances between blood and hepatocytes and between hepatocytes and bile, respectively. Likewise, many transporters expressed in cholangiocyte membranes are also known (Table 2). In this review only those transporters with a clear role in the genesis of physiological bile flow are addressed. More comprehensive reviews are available elsewhere[29-31].

The Golgi complex and the network of microtubules and microfilaments are important structures for the exocrine function of hepatocytes and also for the mechanisms of bile formation. The pericanalicular space, free of cellular organelles, contains actin microfilaments that reach to the microvilli of the canalicular membrane. Microtubules are distributed throughout the cytoplasm. The vesicles from the Golgi complex are vehicles for substances to be excreted in bile as well as plasma proteins, including transporters, to be placed at apical or basolateral membranes[17,32]. Newly synthesized apical

Table 2 Membrane transporters in cholangiocytes: Abbreviations and function

Abbreviation	Function
Basolateral membrane	
NDCBE/SLC4A8[1]	Na^+-dependent Cl^-/HCO_3^- exchanger: Import HCO_3^- and Na^+, Export H^+ and Cl^-
NHE/SLC9[1]	Na^+/H^+ exchanger: Acid extruder
AQP4[1]	Water channel
tASBT/SLC10A2	Export BAs and Na^+
SK2	Potassium channel, potassium efflux
SLC12A2	Import Na^+-K^+-$2Cl^-$
MRP3/ABCC3	Export OA^- conjugates, GSH
MRP4/ABCC4	Export BAs, GSH
Apical membrane	
AE2/SLC4A2[1]	Cl^-/HCO_3^- anion exchanger: Acid loader
CFTR[1]	Cl^- channel: Export chloride
AQP1[1]	Water channel
NBCe/NBC4/SLC4A5	Na^+-HCO_3^- symporter: Acid extruder
ASBT/SLC10A2	BAs-Na^+, symport: Uptake of BAs and Na^+

[1]Transporters relevant to bile flow under physiological basal conditions.

ABC (ATP-binding cassette) transporters are transferred from the Golgi apparatus to the canalicular membrane[16]. This traffic is dependent on intact microtubule and microfilament systems[17,32].

The hepatocytes and the biliary system are closely related to the blood vascular elements, both forming a functional unit: the hepatic acinus[33]. The blood flow generates concentrative gradients of oxygen and nutrients along the sinusoids. These gradients allow a division of the hepatic acinus into three different zones according to its distance from the portal space: the periportal or zone Ⅰ, zone Ⅱ and the centrilobular or zone Ⅲ. Moreover, these gradients cause functional heterogeneity between the hepatocytes of the different zones[34,35].

BILE COMPOSITION

Bile mainly consists of water, in which there are organic and inorganic substances in suspension, dissolved, or in equilibrium between both states. In bile samples, collected from the human common bile duct, the concentrations of the inorganic electrolytes sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and bicarbonate (HCO_3^-) are slightly higher than their plasmatic concentrations, whereas biliary chloride (Cl^-) concentrations are slightly lower than these found in plasma. BAs concentrations range between 2 and 45 mmol/L. The concentrations of biliary pigments range from 50 to 200 mg/100 mL. Proteins and peptides, such as glutathione, are also found in bile[36]. It is also possible to detect glucose and small amounts of endogenous substances such as thyroid and steroid hormones[37]. Human bile is rich in lipids. Thus, phospholipids concentrations seem to range between 25 and 810 mg/100 mL, whereas these of cholesterol vary between 60 and 320 mg/100 mL, with average ratios of phospholipids to BA of 0.3 and cholesterol to BA of 0.07 (Figure 1). Humans differ from other animals in the fact that our species eliminates cholesterol from the body to a greater extent as cholesterol itself rather than by converting it into BAs[38].

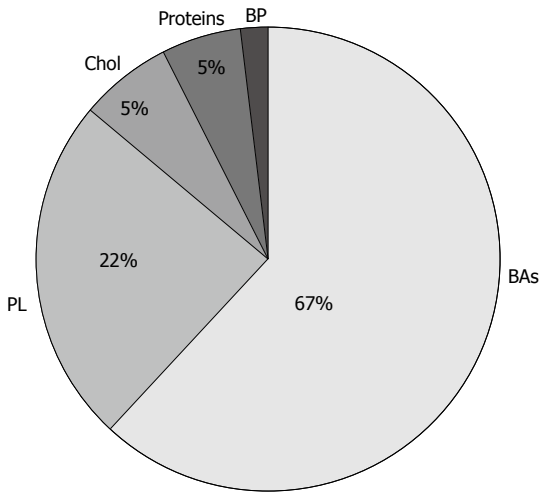

Figure 1 Organic composition of bile. Perceptual distribution of BAs, Phospholipids (PL), Cholesterol (Chol), Proteins and biliary pigments (BP).

BILE FLOW

The mean basal flow of bile in humans is approximately 620 mL/d. One portion of this flow (220 mL/d, 35%) is determined by the secretion of BAs and is called the BAs-dependent canalicular fraction -BADFc-[8]. In addition, there is a linear correlation between the amount of BAs secreted into bile and the amount of water that follows them (7-25 mL/mmol). This choleretic activity of individual BAs is species-dependent and varies according to its chemical structure, conjugational condition and relative concentrations. Choleretic activity is lower for BA species that have a higher tendency to form micellar aggregates in bile[39]. These findings explain the different contributions of BADFc to the bile flow among species (30%-60%)[3,40]. Certain BAs (ursodesoxycholic and the nor-derivatives of ursodesoxycholic and quenodeoxycholic acids) generate a volume of bile higher than that expected from their osmotic force. In order to explain such hypercholeresis, it has been proposed that these BAs would be reabsorbed, in their protonated form, by cholangiocytes. From there, they would be effluxed to blood to reach the sinusoids, where they would be taken up again by hepatocytes and re-secreted to bile, increasing the magnitude of the BADFc[41,42]. This phenomenon, so-called "the cholehepatic shunt pathway" is discussed below in the section devoted to ductular processes.

The amount of canalicular bile independent of the osmotic force of BAs (235 mL/d, 38%) has been designated the BA-independent canalicular fraction (BAIFc)[7,8]. Quantitatively, in humans the BAIFc represents up to a 40% of total primarily formed bile[43]. In other species it varies between 30% and 60%[5,40,44]. The ductular fraction of bile flow has a high value (30%) in humans[25], although it also varies among different species[8,24].

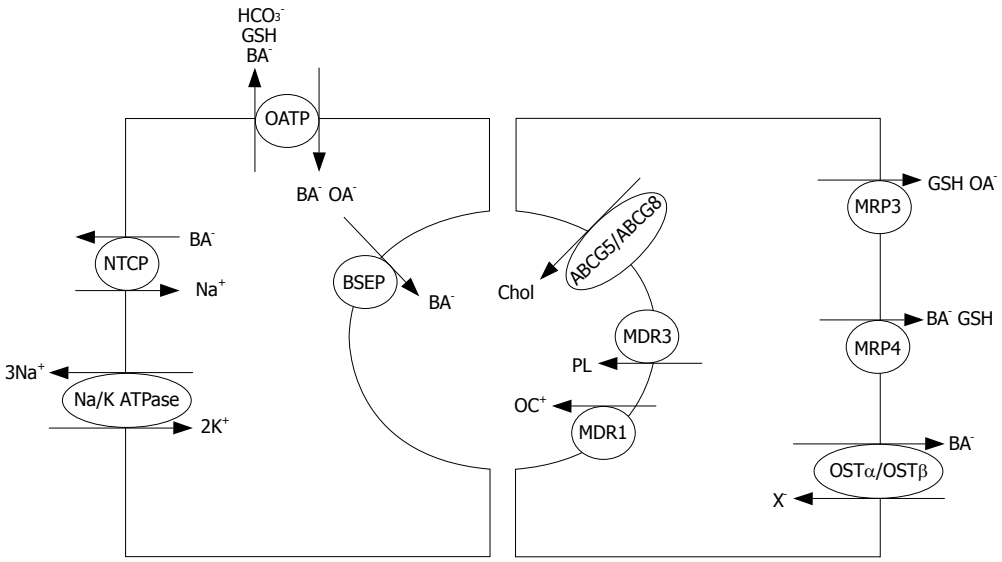

Figure 2 Sinusoidal transport and canalicular secretion. Left: BA dependent canalicular fraction of bile (BADFc). Right: canalicular and sinusoidal secretion of anionic and cationic organic molecules.

THE BA-DEPENDENT CANALICULAR FRACTION (BADFc)

BAs are supplied either by synthesis in liver cells or from the sinusoidal blood as part of *de* enterohepatic circulation (EHC).

Sinusoidal uptake

The BAs in sinusoidal blood are efficiently taken up by hepatocytes from Disse's space despite being highly albumin bound, due to the existence in the basolateral membrane of transporters[45,46]. This uptake is carried out against an electrochemical gradient, is saturable[37] and depends on the structure of the BA. Thus, it is more efficient for trihydroxyl- than for dihydroxyl-BA and for conjugated more than for unconjugated BAs[47]. The sodium taurocholate-cotransporting polypeptide (NTCP), the main Na^+-dependent BAs transporter[30,48] is only expressed in the basolateral membrane[49]. The uptake of BAs by Na^+-independent mechanisms seems to be mediated by less specific transporters, known as organic anion-transporting polypeptides (OATPs), which exchange these molecules for other anions, such as HCO_3^-, glutathione (GSH) or even other BAs[30,47]. These transporters may take up BAs (mainly non conjugated forms), endogenous OA^- (thyroid hormones, monoconjugated bilirubin) and xenobiotic compounds (toxins, drugs, food components, *etc*)[50]. The quantitative relevance of the different isoforms of these transporters in sodium-independent BA uptake by hepatocytes is still not completely understood (Figure 2). The heterodimeric protein OSTalpha/OSTbeta is expressed at the basal membrane of hepatocytes and cholangiocytes[51]. This is a sodium-independent BA transporter that may play a role in BA efflux from hepatocytes toward blood when these compounds get accumulated in cholestatic conditions. Moreover, in cholangiocytes, in addition to play a similar role, this transporter may also be involved in the cholehepatic shunting of BAs.

Transcellular transport

In order to explain the transit of BAs from the sinusoidal membrane to the pericanalicular region, two different, not mutually excluding, mechanisms have been proposed: (1) simple diffusion of BAs bound to intracellular proteins[52]; (2) and/or vesicular transport of BAs driven by cytoskeleton contractile activity[53,54]. Two arguments have been raised against the role of the second mechanism. One is that hepatic transit of labeled BAs is too fast[54]. The second one is that the baseline secretion of BAs is not modified by microtubules disruption[53]. However, the overload of BAs intensify the vesicular trafficking from the Golgi complex to the pericanalicular zone[55], and under these circumstances the alteration in the functional integrity of the cytoskeleton results in impaired BA secretion[56] and subsequently cholestasis[57].

The quantity of ABC transporters in the apical membrane is regulated by the amount of biliary components available for secretion[58,59]. The regulated intracellular vesicular traffic of canalicular ABC transporters[59,60] is crucial for normal bile secretion. The bile salt export pump (BSEP, formerly SPGE, a sister of P-glycoprotein) is the main, if not the only, canalicular BA transporter[61], and it is also located in subcanalicular vesicles that may act as an intracellular pool. It is therefore probable that the impaired secretion of BAs observed in overloaded conditions would be an indirect result of the distortion of the increased vesicular traffic of transporters to the canalicular membrane[56]. These and other studies[17,62] have established not only the actual role of vesicular trafficking in hepatocytes, but also that a specific vesicle trafficking machinery is required for membrane polarity. The overall functions based on hepatocyte polarity are not attributable to the mere presence of transporters in both poles of these cells[63] but also to their intracellular trafficking and temporary anchorage to the different hepatocyte membranes (Figure 2).

Canalicular secretion

At the end of the eighties it was believed that BAs were extruded to the canalicular lumen by an electric gradient, being negative inside hepatocytes (around -37 mV)[64]. However, this gradient is not strong enough

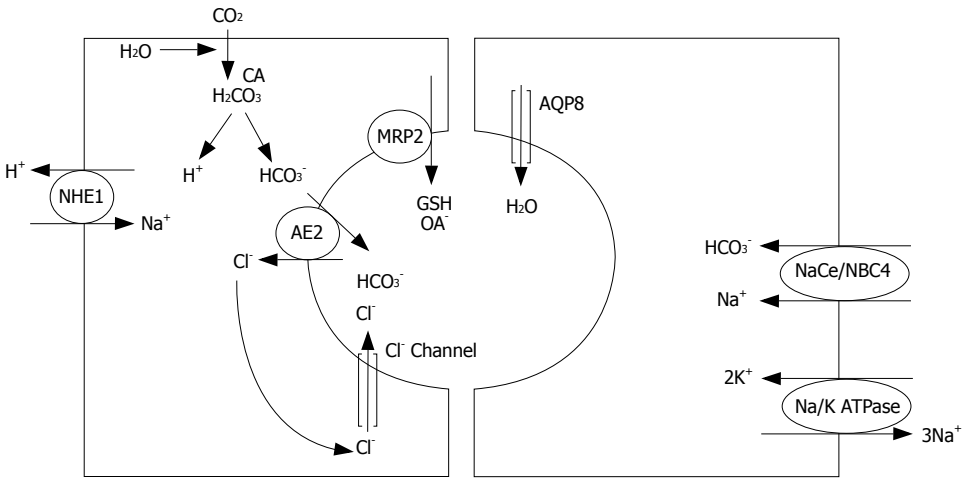

Figure 3 Sinusoidal transport and canalicular secretion. Left: BA independent canalicular fraction of bile (BAIFc). Right: Water an electrolyte movement. CA: carbonic anhydrase.

to impose higher concentrative differences, such as BAs up to 200 times more concentrated in canaliculus than in hepatocyte[26]. It is now known that the secretion of BAs to the canalicular lumen is a saturable phenomenon mediated by a transporter[65]. The energy-dependent bile secretion of BAs is mediated by BSEP[30,58,63]. This export pump was cloned in1998[54] and since them it has been studied in detail[66,67]. It is currently known that BSEP transports both conjugated and unconjugated BAs, sulfated lithocholyl conjugates[68], and a variety of drugs[69]. Other apical ATP binding cassette (ABC) transporters are required: multidrug resistance-P glycoprotein 3 (MDR3) for phospholipids, MDR1 for lipofilic cationic drugs, multidrug resistance-associated protein 2 (MRP2) for non-BA organic anions[70,71] and the heterodimeric protein ABCG5/G8 involved in the secretion of cholesterol and other sterols, such as fitosterols[72] (Figure 2).

BAIFc

The osmotic activity of BAs is not the only determinant of bile flow. Certain substances with osmotic activity, both endogenous and exogenous, may also play a role in canalicular bile generation, without modifying the BA secretion rate[73]. In certain situations, such as diabetes mellitus, there appears to be a reduction in bile flow, without impairment in BA secretion[74]. Glutathione and bicarbonate seem to play a similar quantitative role in BAIFc formation[13].

Multidrug resistance-associated protein 2 (MRP2, formerly cMOAT, canalicular multispecific organic-anion transporter) transports a broad range of amphipathic anionic substrates, including glutathione conjugates, glucuronosyl bilirubin and sulfated BA derivatives[63]. The ATP-dependent canalicular excretion of GSH is one of the main forces responsible for the generation of BAIFc[75]. Under basal conditions, the biliary levels of this tripeptide reache up to 5 mmol/L. This is a sufficient amount to account for the formation of bile by osmotic force[36]. Rats heterozygous and homozygous for inactivating mutations in *Mrp2* secrete less GSH to bile, 37% and 99% below control levels, respectively[76].

The secretion of HCO_3^- is carried out by the canalicular antiporter named AE2[13,77]. This system functions in connection with the canalicular water channel aquaporin (AQP8) and the apical chloride channel, the cystic fibrosis transmembrane regulator (CF^-TR)[13,77]. However, the force that maintains favorable Cl^- gradients remains to be defined. The AE2 antiporter requires the existence of suitable internal levels of HCO_3^- through cotransport of the anion with sodium in the basolateral membrane by the NBCe symporter[78,79] and/or by its formation activated by the carbonic anhydrase (CA) pathway[80]. This latter mechanism is linked to H^+ extrusion *via* Na^+/H^+ antiporters (NHE) working in both the basolateral (NHE1)[81] and canalicular (NHE3)[82] membranes. In turn, sodium cations are extruded *via* a sodium pump. This is why bicarbonate secretion is said to be is a concentrative mechanism that indirectly requires metabolic energy. Canalicular bicarbonate excretion is upregulated by glucagon[83], which also enhances AQP8-mediated water permeability at the canaliculi[84]. These choleretic effects are microtubular-dependent and involve mobilization of intracellular vesicles[83,84]. The other osmotically active inorganic components of bile are not as important as HCO_3^- in generating BAIFc[37,85] (Figure 3).

DUCTULAR PROCESSES

Cholangiocytes exert a series of reabsorptive and secretory processes that dilute and alkalinize the primary (canalicular) bile during its passage along the biliary tract[24,86,87]. Cholangiocytes secrete fluid, HCO_3^-, Cl^- and carry out the reabsorption of glucose, BAs (cholehepatic shunt), glutamate, conjugated bilirubin, BSP, and small OAs^-[87]. As a result, osmotic gradients generate an extra flow of bile known as the ductular BA-independent fraction (BAIFd). These processes are regulated by bile constituents, nerves and hormones[86,88]. The biliary transport of bicarbonate is a relevant function of the cholangiocytes. An electroneutral sodium-independent Cl^-/HCO_3^- exchange activity (AE2) has been observed in the apical membrane of cholangiocytes[77]. There is also a cAMP-responsive Cl^- channel (CFTR) that is coordinated with AE2 to play a role in biliary excretion of HCO_3^-[27,89]. These apical fluxes of anions, in the

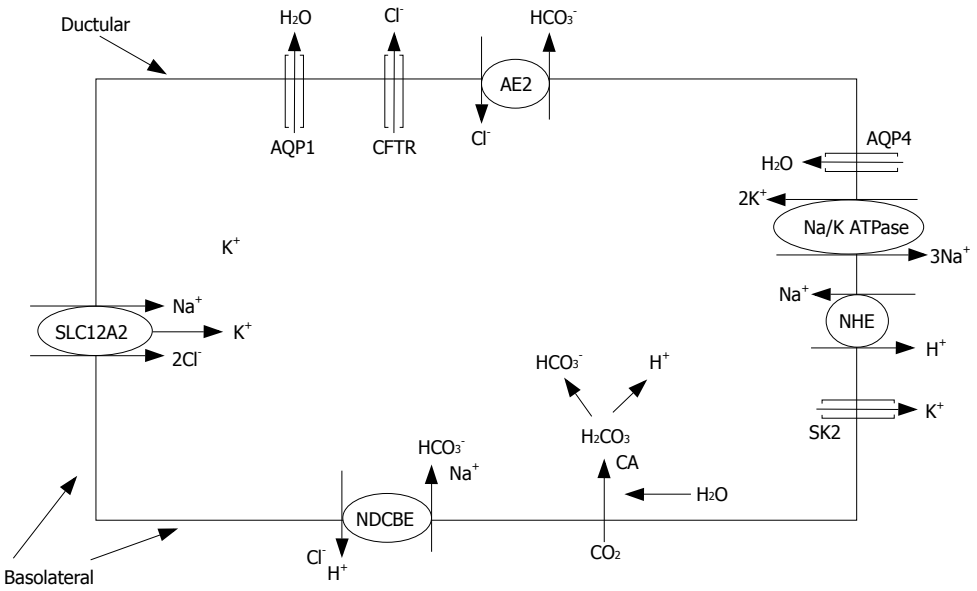

Figure 4 Ductular secretion and reabsorption. BA independent ductular fraction of bile (BAIFd).

presence of aquaporins (AQP1) contribute to the BAIFd[90]. This coordinated function became more evident after discovering that a pool of AE2, CFTR and AQP1 is stored in cholangiocyte intracellular vesicles, which are co-redistributed to the apical membrane under secretin stimulation[90]. The CA pathway and an Cl^-/HCO_3^- exchanger provide the required level of HCO_3^- and the H^+ is subsequently extruded by a coupled carrier-mediated basolateral H^+/Na^+ exchanger (NHE)[91,92]. In humans, the import of HCO_3^- into cholangiocytes occurs mainly through electroneutral Na^+-dependent Cl^-/HCO_3^- anion exchange (NDCBE)[93] (Figure 4).

Lipophilic, unconjugated BAs, such as ursodeoxycholic acids, are passively reabsorbed in cholangiocytes, which constitutes the first essential step in the cholehepatic shunt model[94]. An active transport for conjugated BAs has been described in rat cholangiocytes[95], which expands the idea of cholehepatic shunting of BAS. With the identification of apical (ASBT) and basolateral (tASBT) BAs carriers in cholangiocytes[96], the hypothesis of the cholehepatic BA shunt received additional support[97].

Regulatory factors

Secretin receptors (SCTRs) are exclusively expressed at the basolateral membrane of cholangiocytes[98] and when they are stimulated intracellular levels of cAMP are increased[99]. The pool of AE2, CFTR and AQP1 stored in intracellular vesicles is redistributed to the apical membrane under cAMP or secretin stimulation[90] and secretin stimulation activates CFTR through cAMP. Both effects together explain the increase in HCO_3^- efflux[37]. Most experiments with rats and rabbits have used animals with induced bile duct proliferation[100], since normal rats[23,90] and rabbits[101] respond very poorly to secretin. ASBT activity increases acutely upon secretin stimulation[102], which may accentuate the cholehepatic BAs shunting in the postprandial period.

On cholangiocytes, acetylcholine increases both secretin-stimulated cAMP synthesis and Cl^-/HCO_3^- exchanger activity[103,104]. Vagotomy in BDL rats inhibits secretin-stimulated ductal secretion and decrease cholangiocyte cAMP levels[88]. Bombesin can act either by increasing the secretin release in dogs[105], or inducing ductal secretion with activated Cl^-/HCO_3^- exchange *via* secretin-independent mechanisms in isolated rat cholangiocytes[86]. VIP increases secretin-stimulated bile flow and HCO_3^- excretion in humans[106]. Dopamine, somatostatin and, gastrin to some extent, inhibit basal and secretin-stimulated bicarbonate-rich choleresis[86,107,108].

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