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CALCIUM SIGNALING AND THE SECRETORY ACTIVITY OF BILE DUCT EPITHELIA

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Abstract

Cytosolic calcium (Ca_i^{2+}) is a second messenger that is important for the regulation of secretion in many types of tissues. Bile duct epithelial cells, or cholangiocytes, are polarized epithelia that line the biliary tree in liver and are responsible for secretion of bicarbonate and other solutes into bile. Ca_i^{2+} signaling plays an important role in the regulation of secretion by cholangiocytes, and this review discusses the machinery involved in the formation of Ca^{2+} signals in cholangiocytes, along with the evidence that these signals regulate ductular secretion. Finally, this review discusses the evidence that impairments in cholangiocyte Ca^{2+} signaling play a primary role in the pathogenesis of cholestatic disorders, in which hepatic bile secretion is impaired.

1. Introduction

Numerous cellular functions are regulated by cytosolic calcium (Ca_i^{2+}) [1, 2]. In the liver, Ca_i^{2+} controls such diverse processes as glucose and energy metabolism, cell proliferation, apoptosis, and bile secretion. This complex simultaneous regulation results from highly organized temporal Ca^{2+} signaling patterns, such as Ca^{2+} spikes and oscillations, and spatial signaling patterns, such as Ca^{2+} gradients and waves [3]. In both hepatocytes and cholangiocytes, these properties of Ca^{2+} signals are mediated entirely by inositol 1,4,5-trisphosphate (InsP3), which binds to InsP3 Receptors (InsP3Rs) to promote Ca^{2+} release from the Endoplasmic Reticulum (ER) [3, 4]. Ca^{2+} signals in both of these types of epithelia are not only organized at the cellular level, but are also integrated in the whole organ through a signaling network that depends on gap junctions [5, 6] and paracrine messengers [7–9] to establish intercellular communication.

Many aspects of Ca^{2+} signaling are important in the various cell types in the liver [3]. This review will describe the cellular machinery that generates Ca^{2+} signals in cholangiocytes,

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Conflict of interest statement

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the role of Ca^{2+} signals in the secretory activity of these cells, and their involvement in liver health and disease.

2. Mechanisms of Ca_i^{2+} signaling

2.1 Molecular machinery for Ca^{2+} signal formation in cholangiocytes

There are two general mechanisms of Ca_i^{2+} signal formation: Ca^{2+} influx across the plasma membrane (PM) and Ca^{2+} release from intracellular stores. Several second messengers elicit Ca_i^{2+} release from intracellular stores, largely through binding to specific intracellular receptors and the regulation of their activity [1, 2]. In cholangiocytes, InsP3 is the predominant intracellular Ca^{2+} -mobilizing messenger [10], which binds to the InsP3R, the main Ca^{2+} release channel in epithelia, and the only intracellular Ca^{2+} release channel found in cholangiocytes [11]. InsP3 is generated through the stimulation of either PM G-protein-coupled receptors (GPCRs) by Ca^{2+} -mobilizing hormones, or receptor tyrosine kinases (RTKs) by growth factors [1, 12].

Stimulation of GPCRs leads to the activation of phospholipase C (PLC), which hydrolyses phospholipid phosphatidylinositol-4-5-bisphosphate (PIP2) within the PM generating diacylglycerol (DAG) and InsP3. DAG interacts with protein kinase C (PKC) at the PM while InsP3 diffuses into the cytoplasm to bind to InsP3Rs, which allow the release of Ca^{2+} from intracellular stores [1]. Activation of RTKs is thought to similarly promote PLC-mediated PIP2 hydrolysis at the PM. However, recent evidence suggests that RTK-mediated PLC activation may alternatively result in hydrolysis of nuclear PIP2 and subsequent Ca^{2+} release within the nucleoplasm [13–15]. This alternative pathway is of demonstrated importance in liver cell lines, primary hepatocytes, and intact liver [13–15]. InsP3Rs are commonly found in the membrane of the ER [12] and the nuclear envelope (NE) [16], although they have been observed in the plasma membrane of certain cell types [17], as well as along the nucleoplasmic reticulum [16].

There are three InsP3R isoforms (types I, II and III), each of which acts as an InsP3-gated Ca^{2+} channel with distinct biophysical properties [18]. Cells can express different InsP3R isoforms, and some cell types [19, 20], including cholangiocytes [11], express all three isoforms. There can be considerable variability among different cell and tissue types in the expression levels of each isoform and in their subcellular distribution. In cholangiocytes, the type III InsP3R isoform accounts for approximately 80% of InsP3Rs, while types I and II each account for about 10%. In addition, type III InsP3R is most concentrated in the apical region (Figure 1a), while the other isoforms are dispersed relatively uniformly throughout the cytoplasm in a non-polarized manner [11]. This apical distribution of the type III InsP3R is likely responsible for triggering polarized (apical-to-basolateral) Ca^{2+} waves in cholangiocytes [11], similar to what is observed in other polarized epithelia [21–23], including hepatocytes [24]. Although there is significant morphological and functional heterogeneity between small and large cholangiocytes [25], both of these cell types can signal through InsP3/InsP3Rs (see below) [8, 11, 25, 26].

An alternative mechanism for Ca^{2+} release from the ER is through ryanodine receptors (RyRs). These channels can be activated in several ways, including direct coupling with

certain plasma membrane Ca^{2+} channels, interaction with the second messenger cyclic ADP ribose, or stimulation by Ca^{2+} itself (Ca^{2+} -induced Ca^{2+} release, or CICR) [1, 27]. RyRs are most commonly found in excitable cells but are also expressed in certain polarized epithelia, such as pancreatic acinar cells [21]. However, there is no evidence for fully functional RyRs in cholangiocytes or hepatocytes [11, 28], although a truncated RyR may be present in hepatocytes [28].

An additional second messenger that can promote Ca^{2+} release into the cytosol is nicotinic acid adenine dinucleotide phosphate (NAADP), which in fact is the most potent Ca^{2+} mobilizing messenger yet identified [29]. NAADP is thought to interact with two-pore channels (TPCs) to promote Ca^{2+} release from acidic stores [30, 31], although accessory proteins within a larger molecular complex seem to play a role in this process [32]. There are three isoforms of TPCs, 1, 2 and 3, although only 1 and 2 are present in mammals [30]. TPCs display differential subcellular distribution. TPC2, in particular, is predominantly expressed in lysosomes and has been detected in many human tissues, including the liver [30]. However, whether TPCs are present in cholangiocytes and their role in the physiological functions of these cells remain to be determined.

2.2 Basolateral and apical plasma membrane receptors that signal through Ca^{2+}

InsP3-mediated Ca^{2+} signals in cholangiocytes result from the interaction between various Ca^{2+} -mobilizing hormones and specific GPCRs localized in either the basolateral or the apical membrane [33]. For instance, M3 muscarinic receptors, which are found on the basolateral membrane, are activated by the neurotransmitter Acetylcholine (ACh) [34]. Stimulation of cholangiocytes with ACh can result in a sustained, transient or oscillatory increase in Ca_i^{2+} [10]. Furthermore, ACh promotes apical-to-basolateral Ca^{2+} waves (Figure 2) [11], which play an essential role in the regulation of ductular bicarbonate secretion (see below) [34]. Adenosine triphosphate (ATP) also mediates Ca_i^{2+} release in cholangiocytes, through interaction with plasma membrane nucleotide receptors. Pharmacological evidence from isolated rat intrahepatic bile duct units suggests that P2Y1, P2Y2, P2Y4 and P2Y6 are found on the apical membrane [35]. Although P2Y receptors are also expressed on the basolateral membrane, their activity is attenuated by local nucleotide hydrolysis [35] or by surrounding portal fibroblasts [36]. P2X receptors, which are ATP-gated Ca^{2+} -permeable cation channels, are also expressed in cholangiocytes [35, 37]. Like ACh, ATP stimulation induces a sustained, transient or oscillatory increase in Ca_i^{2+} [10]. In addition, ATP promotes the formation of apical-to-basolateral Ca^{2+} waves, although these spread more slowly than ACh-induced Ca^{2+} waves [11]. Although there are certain similarities between ATP- and ACh-induced Ca^{2+} signaling patterns, lower concentrations of ATP result in Ca^{2+} oscillations, whereas higher concentrations cause single (either sustained or transient) increases in Ca^{2+} . On the other hand, the occurrence of ACh-induced Ca^{2+} oscillations is not dose-dependent [10]. Furthermore, Ca_i^{2+} spikes within ATP-induced Ca_i^{2+} oscillations display more regular frequency and width than ACh-induced oscillations. Finally, the period of Ca_i^{2+} oscillations induced by ATP is shorter than that induced by ACh [10]. ATP can be secreted by hepatocytes [9] and cholangiocytes [7, 8], allowing this messenger to regulate Ca_i^{2+} signals in an autocrine and paracrine fashion [38]. This autocrine/paracrine signaling is important for cell volume regulation [39] and bicarbonate secretion [8, 40].

Cholangiocytes also express receptors for a number of Ca_i^{2+} -mobilizing biogenic amines [41]. The H1HR histamine receptor, found in the basolateral membrane, mobilizes Ca_i^{2+} signals in small cholangiocytes through InsP3/InsP3R, regulating their proliferation in an InsP3/CAMK I/CREB-dependent manner [42, 43]. Other histamine receptor subtypes are found in cholangiocytes and are linked to the control of cell proliferation as well (H2HR, H3HR and H4HR). However, their actions involve the stimulation of cyclic adenosine monophosphate-(cAMP) rather than InsP3/ Ca^{2+} -dependent signaling mechanisms [41, 43]. Furthermore, serotonin receptors (5-HT 1A and 1B) are found on the basolateral membrane and their activation inhibits cholangiocyte proliferation, secretin-stimulated cAMP synthesis and protein kinase A (PKA) activity, hence suppressing ductular secretion. These effects are associated with enhanced InsP3 levels, increased Ca^{2+} -dependent PKC activity and reduced cAMP/PKA pathway [44]. Similarly, D2 dopamine receptor activation inhibits secretin-stimulated cAMP production and suppresses cholangiocyte proliferation through Ca^{2+} -dependent activation of PKC γ [45]. Finally, selective activation of basolateral α_1 -adrenergic receptors by phenylephrine increases secretin-stimulated ductular secretion by Ca^{2+} -dependent activation of PKC- α and PKC- β II and consequent amplification of the cAMP signaling pathway [46]. Moreover, phenylephrine stimulates small cholangiocyte proliferation through the Ca^{2+} -dependent activation of nuclear factor of activated T cells 2 (NFAT2) and specificity protein 1 (Sp1) [47]. Table 1 summarizes the physiological effects of the various agonists and their respective plasma membrane receptors.

Therefore, cholangiocytes possess a wide array of basolateral and apical receptors that act through Ca_i^{2+} . However, activation of the various receptors can elicit distinct types of downstream responses, a feature that is closely related to their differential effects on Ca_i^{2+} signaling patterns and the activation of specific intracellular signaling pathways.

2.3 Intercellular communication

Ca^{2+} signals in cholangiocytes are coordinated not only at the single cell level, but are also transmitted to neighboring cells [5, 7–9]. Gap junctions play an important role in this process and are, in fact, permeable to both InsP3 and Ca^{2+} [48]. Cholangiocytes express predominantly the gap junctional protein connexin 43, which synchronizes Ca^{2+} oscillations in these cells. Moreover, permeability of cholangiocyte gap junctions is under hormonal control and is decreased by the activation of PKA or PKC [5].

Paracrine signaling is also important for intercellular communication in cholangiocytes. This is possible through the action of such extracellular messengers as ATP. This agonist is secreted into bile by both hepatocytes [9] and cholangiocytes [7, 8] and interacts with apical G-protein coupled P2Y receptors expressed in both of these cell types [35, 49]. Therefore, when released into bile by hepatocytes, ATP promotes InsP3-dependent Ca_i^{2+} release in neighboring hepatocytes as well as in cholangiocytes that are downstream [9]. Notably, both small and large cholangiocytes participate in this purinergic-signaling axis. Therefore, “upstream” cholangiocytes lining the small intrahepatic bile ducts can secrete ATP into the bile, which acts as a paracrine messenger in “downstream” large cholangiocytes to similarly induce Ca_i^{2+} release [26]. In addition, ATP released from cholangiocytes can act in an autocrine manner to stimulate apical P2Y receptors [8]. This paracrine/autocrine signaling

system allows the propagation of Ca^{2+} signals to adjacent cells or within individual cells independent of gap junctional communication.

3. Ca^{2+} signals and cholangiocyte secretory activity

Cholangiocytes are responsible for conditioning of the canalicular bile that is secreted by hepatocytes [50]. Although these cells account for only 3–5% of the total liver mass, they play an essential role in the overall process of normal bile secretion, [50, 51] and do so by regulating the concentration of biliary bicarbonate [52]. Bicarbonate secretion occurs through the action of two main signaling pathways. The first is initiated by the stimulation of basolateral secretin receptors, which results in production of cAMP and activation of the apical chloride channel cystic fibrosis transmembrane conductance regulator (CFTR), following phosphorylation by PKA. The release of chloride in the ductular lumen is thought to provide the driving force for chloride-bicarbonate exchange, which mediates bicarbonate secretion into the bile [53]. The second pathway is mediated by InsP3 -dependent Ca^{2+} release and is activated by neurotransmitters like ACh or by paracrine agents like ATP. Ca^{2+} acts directly on apical Ca^{2+} -dependent chloride channels, recently identified as TMEM16A channels in both small and large cholangiocytes [54], allowing the efflux of chloride, which is then similarly exchanged for bicarbonate via the chloride-bicarbonate exchanger [53, 55]. These two pathways were initially thought to act independently, but evidence that Ca^{2+} enhances adenylyl cyclase activity and cAMP formation through a calcineurin-dependent pathway suggests that there may be a cross-talk, and in fact, an indirect mechanism for Ca^{2+} -mediated bicarbonate secretion [34]. Moreover, further studies revealed that there might be a closer connection between Ca^{2+} and cAMP/CFTR-mediated secretion. cAMP production leads to apical, CFTR-dependent release of ATP into the bile, which activates apical P2Y receptors to induce InsP3 -mediated Ca^{2+} release through apical type III InsPRs . Ca^{2+} then drives apical Ca^{2+} -dependent chloride channel activation and subsequent bicarbonate secretion (Figure 3) [8, 40]. Thus, CFTR and TMEM16A act in concert to regulate biliary secretion [54], similar to what has been observed in pancreatic duct epithelial cells [56]. These observations place cAMP as the intracellular trigger for an autocrine signaling loop in which bicarbonate secretion is mediated by ATP production, activation of P2Y receptors and Ca_i^{2+} release. In addition, this might indicate that InsP3 -mediated Ca^{2+} release represents a convergence point for secretory pathways in the cholangiocyte [8]. On one hand, stimulation of basolateral M3 receptors by ACh increases InsP3 , which activates basolateral types I and II InsP3Rs to release Ca^{2+} . Ca^{2+} in turn activates apical Ca^{2+} -dependent chloride channels, leading to apical bicarbonate secretion. On the other hand, activation of basolateral secretin receptors results in cAMP production, and then apical CFTR-dependent release of ATP, which then also leads to Ca^{2+} -mediated bicarbonate secretion (Figure 3) [8]. Although ACh induces the formation of apical-to-basolateral Ca^{2+} waves [11], the mechanism by which this process occurs is not entirely understood because ACh-induced InsP3 formation presumably occurs in the basolateral region. One possibility is that the basolateral component of ACh-induced Ca^{2+} signals is more important for bicarbonate secretion through the activation of types I and II InsP3Rs , although Ca^{2+} waves may nonetheless begin apically because of clustering of type III InsP3Rs there. In order to understand possible differences between Ca^{2+} waves and

bicarbonate secretion induced by ACh as opposed to ATP, further studies using low affinity Ca^{2+} dyes to quantify the apical Ca^{2+} concentration or patch clamp measurements of chloride or bicarbonate conductance may need to be carried out.

Fluid flow (or shear stress) induces mechanosensitive release of ATP and Ca^{2+} activation of non-CFTR chloride channels [57].

Together with the above considerations, these findings suggest that purinergic signaling may act in concert with, or even potentiate secretin-mediated bicarbonate secretion through CFTR [40]. A main role of CFTR, in fact, might be to regulate ATP release rather than to secrete chloride and/or bicarbonate [8].

Notably, both small and large cholangiocytes utilize Ca^{2+} signals to regulate biliary bicarbonate secretion. In fact, small cholangiocytes are also known as Ca^{2+} -dependent cholangiocytes, since they lack expression of secretin receptors or CFTR. Therefore, GPCR stimulation by ACh or ATP leads to Ca^{2+} -dependent chloride channel activation and bicarbonate secretion [25]. Large cholangiocytes, on the other hand, are denoted cAMP-dependent cholangiocytes, and signal predominantly through cAMP, although GPCR activity also induces Ca^{2+} -dependent bicarbonate secretion [25]. Therefore, although there is heterogeneity between these two cell types, Ca^{2+} signaling acts as a unifying mechanism to regulate secretion. In particular, purinergic signaling is important in both small and large cholangiocytes, where ATP released by hepatocytes and cholangiocytes acts in small and large cholangiocytes to stimulate Ca^{2+} -dependent bicarbonate secretion [26], through the regulation of TMEM16A activity [54].

Ca^{2+} signaling and secretion can also be regulated by primary cilia, which are sensory organelles that extend from the cholangiocyte apical membrane into the ductal lumen. Primary cilia can detect and transmit mechanical stimuli from within the bile duct lumen, so that changes in bile flow affect both cAMP production and Ca^{2+} release [58]. In addition, changes in osmolarity can be sensed by these organelles through activation of the Ca^{2+} -permeable ion channel transient receptor potential channel vanilloid subfamily 4 (TRPV4). This allows entry of extracellular Ca^{2+} into the cytoplasm, ATP release and bicarbonate secretion [59].

The Ca^{2+} -activated small conductance K^{+} channel SK2, has been identified and characterized in biliary epithelia as an additional mediator of Ca^{2+} -dependent chloride secretion. This channel appears to maintain the electrical driving force for continued chloride efflux, thus regulating bicarbonate secretion [60].

The apical localization of type III InsP3Rs in cholangiocytes has critical implications for bile secretion. Clustering of this isoform close to the apical membrane may allow for the formation of a signaling microdomain, where III InsP3Rs act as a molecular trigger for Ca^{2+}_i signals which in turn increase this isoform's sensitivity [61–63]. This may allow relatively high Ca^{2+} concentrations to be reached in the apical region, which might be important for the insertion of key membrane proteins for bile secretion such as water transporters [64]. This is supported by evidence that subplasmalemmal Ca^{2+} signals generally are obligatory for exocytosis [65, 66], Ca^{2+} acts as a regulator of exocytosis in the

liver in particular [67], and apical Ca^{2+} , released from apical type II InsP3Rs, regulates the insertion of the transporters Multidrug resistance associated protein 2 (MRP2) and bile salt export pump (BSEP) in the apical membrane of hepatocytes [68, 69]. Moreover, apical type III InsP3Rs in cholangiocytes trigger apical-to-basolateral Ca^{2+} waves [11], which are known to regulate fluid and electrolyte secretion in other polarized epithelial cells [68–70]. Because Ca^{2+} mediates bicarbonate secretion via apical Ca^{2+} -dependent chloride channels, polarized Ca^{2+} waves could potentially direct secretory activity in cholangiocytes as well [11].

4. Ca^{2+} signals in cholangiocytes in health and disease

Bile secretion is one of the cardinal functions of the liver. Defects in this process lead to cholestasis, a condition that is among the most serious manifestations of liver disease, and generally results from impaired bile duct function [51, 71]. Given the close relationship between Ca^{2+} and the regulation of bile secretion, the effects of defects in the Ca^{2+} signaling machinery on the pathogenesis of cholestasis have been investigated [52, 64]. Loss of InsP3R isoform expression is a common event in cholestatic conditions [72]. In particular, apical type III InsP3R expression is markedly decreased or absent in the bile ducts of patients with a range of cholestatic disorders including bile duct obstruction resulting from gallstone disease or malignancy, biliary atresia, primary biliary cirrhosis and sclerosing cholangitis. Similarly, selective loss of bile duct InsP3R expression is observed in animals subjected to common bile duct ligation (BDL) (Figure 1b) or lipopolysaccharide (LPS) injection, which are accepted models of ductular cholestasis [71, 72], and is associated with impaired Ca^{2+} signaling and Ca^{2+} -mediated bicarbonate secretion. However, there is no change in upstream components of the Ca^{2+} signaling pathway in these conditions. This suggests that the defects in Ca^{2+} signaling are specifically due to selective decrease in InsP3R expression [72]. Furthermore, because cAMP-mediated ductular secretion also relies on Ca^{2+} -mediated Ca^{2+} release, loss of InsP3R expression might affect this process as well [8, 46]. Interestingly, InsP3R expression is normal in the bile ducts of patients infected with Hepatitis C virus; this viral infection induces inflammation in the region of the bile ducts, although the virus typically damages hepatocytes but not bile duct epithelia, and does not generally result in cholestasis. This suggests that loss of InsP3Rs from cholangiocytes is specific for cholestatic conditions and is not merely the effect of local peri-portal inflammation or injury [72]. The molecular mechanisms underlying the decrease of InsP3R expression remain to be investigated. Accumulation of bile acids in cholestasis can lead to decreased expression of certain hepatic proteins through the activation of nuclear hormone receptors such as the farnesoid X receptor (FXR) [73]. However, it is not known whether any of the InsP3R isoform gene promoters contain elements that are sensitive to FXR. Proinflammatory cytokines released during cholestasis are known to inhibit fluid secretion in cholangiocytes [74], so their involvement in decreased expression of InsP3R is plausible as well. However, there is evidence that proinflammatory cytokines do not impair Ca^{2+} -mediated secretion in these cells [74]. In fact, IL-4 upregulates TMEM16A expression leading to increased transepithelial secretion [54]. Alternatively, loss of InsP3R expression could be explained by increased degradation rather than decreased production. In fact, secretagogue stimulation in pancreatic acinar cells leads to rapid InsP3R ubiquitination and

proteasomal degradation [75]. Thus, decreased production, increased degradation or both may contribute to the decreased InsP3R expression in cholestatic cholangiocytes [72].

These observations beg the question that, in the same way that impaired Ca^{2+} signals are involved in the development of cholestatic conditions, can enhancement of Ca^{2+} signals in cholangiocytes be of therapeutic benefit? Relevant to this question is the observation that ursodeoxycholic acid (UDCA), a hydrophilic bile acid which is the only therapy of proven efficacy for certain types of chronic cholestatic liver disease [76, 77], stimulates biliary Ca^{2+} -dependent exocytosis to enhance liver function [78, 79]. Furthermore, enhancement of Ca^{2+} signaling mediates the cytoprotective effects of UDCA and its taurine conjugate TUDCA [80]. In fact, UDCA stimulates secretion of ATP from hepatocytes into bile, which may promote bile flow and act on downstream bile duct epithelia by stimulating Ca^{2+} -dependent chloride and bicarbonate secretion [81]. Additionally, UDCA can locally stimulate cholangiocytes to release ATP and initiate P2Y receptor-mediated Ca^{2+} -dependent bicarbonate secretion [7]. Therefore, stimulation of Ca^{2+} signaling in cholangiocytes may be an efficient strategy for the treatment of cholestatic diseases.

Knockout mice have been generated for all three InsP3R isoforms [82, 83]. Type II and III knockout animals, in particular, have deficient secretion of saliva, pancreatic zymogens [82] and olfactory mucus [84]. However, these animals display no obvious liver defects [82] and whether biliary secretion is impaired has not been carefully investigated.

Gap junctional communication is also implicated in the maintenance of liver health. In fact, deficiency of connexin 32, the most abundant gap junctional protein in the liver [85, 86], leads to impaired intercellular communication and defective coordination of Ca^{2+} signals in hepatocytes after endotoxin treatment [87], as well as prevents the amplification of liver injury after acetaminophen-induced hepatotoxicity [88]. Furthermore, connexin 32 deficient animals have prolonged cholestasis when compared to wild type animals, indicating that the expression of this protein is important for the recovery from endotoxin-induced cholestasis [87]. Similarly, cholestatic but not choleretic bile acids inhibit the permeability of gap junction channels in both hepatocytes and cholangiocytes [89]. This leads to impaired coordination of Ca^{2+} oscillations in hepatocytes in particular [89], but could potentially affect Ca^{2+} signal organization in cholangiocytes as well, and hence, their ability to control their secretory activity. This inhibition might contribute to the cholestatic effects of these bile acids. Therefore, Ca^{2+} signaling defects are involved in the pathogenesis of cholestasis, and maintenance of Ca^{2+} homeostasis appears to be important for therapeutic success.

5. Conclusions

The versatility and specificity of Ca^{2+} signals in cholangiocytes results from stimulation by a variety of apical and basolateral hormones and neurotransmitters and relies on the concerted action of a number of molecular components within the cell. The apical concentration of type III InsP3Rs appears to be of particular importance, both for the formation of polarized Ca^{2+} signals and for ductular bicarbonate secretion. Moreover, cholestasis appears to be one of the few examples of human diseases that result directly from loss of (type III) InsP3Rs. Understanding how Ca^{2+} signals regulate liver health and how

defects may lead to disease states is of great importance to find new, more targeted and effective therapies, and to improve the actions of the ones that are already available.

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Figure 1.

Type III InsP3R is concentrated in the apical region of bile duct epithelia and is lost after bile duct ligation. Confocal immunofluorescence of liver sections from rats before and after bile duct ligation (BDL) and double-labeled with a type III-specific InsP3R antibody (green) and rhodamine phalloidin (red) to identify actin beneath the plasma membrane. Type III InsP3R labeling is localized mainly in the apical region of bile duct cells in normal liver sections (A) and is greatly reduced 2 weeks after BDL (B). (Modified from reference [72] with permission).

Figure 2.

Ca^{2+} waves begin in the apical region of cholangiocytes. (A) Confocal image of a portal tract segment loaded with the lipophilic dye 1,1-dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine perchlorate (DiI) to facilitate identification. (B) Confocal image of the same segment loaded with the Ca^{2+} -sensitive dye fluo-4/AM. The white line represents the portion of the segment used to visualize Ca^{2+} waves by confocal line scanning microscopy. The line crosses the apical-to-basal axis of one of the cells. This and the subsequent image are pseudocolored according to the color scale shown at the bottom. (C) Confocal line scanning image of the cell shown in B after stimulation with ACh. A polarized Ca^{2+} wave is observed as an increase in fluorescence that starts at the apical region and then spreads rapidly to the basal region. (D) Graphical representation of the fluorescent increase shows that the apical Ca^{2+} signal precedes the basal Ca^{2+} signal. (modified from reference [11], with permission).

Figure 3.

Dependence of cAMP/CFTR-mediated bicarbonate secretion on Ca²⁺ signaling pathways in cholangiocytes. Stimulation of basolateral secretin receptors results in cAMP increase, which induces apical, CFTR-dependent release of ATP. This stimulates apical P2Y nucleotide receptors, which promote local InsP3 production and Ca²⁺ release from apical type III InsP3Rs, leading to Ca²⁺-dependent chloride efflux and apical bicarbonate secretion. (modified from reference [8] with permission).

Table 1Physiological effects of agonists/plasma membrane receptors that act through Ca^{2+} in cholangiocytes

Agonist/Plasma Membrane Receptor	Localization	Physiological effects	Mechanism of action	References
ACh/M3 muscarinic receptor	Basolateral membrane	Stimulation of ductular HCO_3^- secretion	Apical-to-basolateral Ca^{2+} waves; activation of apical Ca^{2+} -dependent Cl^- channels; potentiation of secretin effect on $\text{Cl}^-/\text{HCO}_3^-$ exchanger activity	[8, 10, 11, 34]
ATP/P2Y receptors	Predominantly apical membrane	Regulation of cell volume; Stimulation of ductular HCO_3^- secretion	Apical-to-basolateral Ca^{2+} waves; activation of apical Ca^{2+} -dependent Cl^- channels	[8, 10, 11, 39, 40]
Histamine/H1HR	Basolateral membrane	Stimulation of small cholangiocyte proliferation	Activation of $\text{InsP}_3/\text{CAMK I}/\text{CREB}$ pathway	[42, 43]
Serotonin/5-HT 1A and 1B receptors	Basolateral membrane	Inhibition of secretin-stimulated ductular secretion; suppression of cholangiocyte proliferation	Increase of Ca^{2+} -dependent PKC activity	[44]
Dopamine/D2 receptor	Basolateral membrane	Inhibition of secretin-stimulated ductular secretion; suppression of cholangiocyte proliferation	Increase of Ca^{2+} -dependent PKC γ activity	[45]
Phenylephrine/ α 1-adrenergic receptor	Basolateral membrane	Potentiation of secretin-stimulated ductular secretion; stimulation of small cholangiocyte proliferation	Ca^{2+} -dependent activation of PKC- α and PKC- β II; Ca^{2+} -dependent activation of NFAT2 and Sp1	[46, 47]