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Calcium signaling and secretion in cholangiocytes

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Abstract

Alcoholic hepatitis affects up to one-third of individuals who abuse alcohol and can be associated with high mortality. Although this disorder is characterized by hepatocellular damage, steatosis and neutrophil infiltration, recent evidence suggests that cholestasis or impaired bile secretion may be a frequent occurrence as well. Bile secretion results from the concerted activity of hepatocytes and cholangiocytes, the epithelial cells that line the bile ducts. Hepatocytes secrete bile acids and conjugated products into the bile canaliculi, which then are modified by cholangiocytes through secretion of bicarbonate and water to give rise to the final secreted bile. Here the molecular mechanisms regulating bile secretion in cholangiocytes are reviewed. Moreover, we discuss how the expression of intracellular Ca^{2+} channels might be regulated in cholangiocytes, plus evidence that components of the Ca^{2+} signaling machinery are altered in a range of cholestatic diseases of the bile ducts.

Keywords

Bile ducts; alcoholic hepatitis; cholestasis; InsP3 receptors

Introduction

Bile secretion in the liver involves the combined activities of hepatocytes and cholangiocytes[1]. Bile acids and conjugated excretion products along with cholesterol and other lipids are secreted by hepatocytes through specific transporters localized at the apical (canalicular) membrane and modified by the secretory activity of cholangiocytes, mostly through bicarbonate (HCO_3^-) and water secretion to the lumen of bile ducts. A range of liver diseases that are characterized by cholestasis, or impaired bile secretion, result from defective secretory activity of cholangiocytes rather than hepatocytes. These disorders include primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), biliary atresia, bile duct obstruction, and cystic fibrosis liver disease (CFLD)[2]. Although jaundice is commonly observed in alcoholic liver disease, it has typically been attributed to

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hepatocellular damage rather than to defective secretion. However, several recent lines of evidence suggest that cholestasis can occur in alcoholic hepatitis and may in fact be associated with a worse outcome[3, 4]. In this review we discuss the basic mechanisms that regulate fluid secretion in cholangiocytes, plus recent evidence implicating novel regulators of Ca^{2+} signaling in the pathogenesis of ductular cholestasis.

Signaling mechanisms in biliary secretion

The secretory activity of cholangiocytes is regulated by both cAMP and Ca^{2+}_i signaling pathways[5]. The prototypical example of cAMP-dependent fluid secretion in cholangiocytes is the secretin receptor pathway. These receptors are localized at the basolateral membrane of cholangiocytes and upon binding of secretin, trigger formation of intracellular cAMP. This second messenger activates protein kinase A (PKA), which phosphorylates and activates the cystic fibrosis transmembrane conductance regulator (CFTR) at the apical membrane, promoting Cl^- secretion into the lumen of the bile ducts[6]. This Cl^- efflux creates the driving force for the secretion of HCO_3^- via activation of the anion exchanger 2 (AE2), the protein encoded by the SLC4A2 gene, the net result of which is the alkalinization of bile[7]. An additional contribution stems from the activation of apical Ca^{2+}-activated Cl^- channels. These channels have been recently identified as the product of the TMEM16A gene and promote efflux of Cl^- in response to elevations of intracellular Ca^{+2} and reach their maximum current at $[Ca^{2+}_i]$ of about 5 μM[8].

Multiple mechanisms contribute to increases in intracellular Ca^{2+} in cholangiocytes. First, experiments on primary mouse cholangiocytes, isolated intrahepatic bile duct units (IBDU) and microdissected intrahepatic bile duct segments have shown that muscarinic receptor activation promotes fluid secretion[9–11]. Acetylcholine or its analog carbachol binding to M3 muscarinic receptors on the basolateral membranes promotes formation of inositol 1,4,5-trisphosphate (InsP3) and diacylglycerol (DAG) from the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C (PLC). InsP3 diffuses through the cytoplasm and binds to the InsP3 receptors (InsP3R) on the surface of the endoplasmic reticulum (ER) leading to the release of Ca^{2+} from the ER lumen to the cytosol. Second, activation of purinergic nucleotide receptors on the apical membrane of cholangiocytes represents a major driver of bile duct secretion. Similar to muscarinic receptors, binding of ATP or UTP to P2Y receptors (P2YR) leads to an InsP3-dependent Ca^{2+} release from intracellular stores[10, 12]. This unique mechanism requires the presence of ATP in the lumen of bile ducts, the source of which is still unclear[13]. Experimental evidence has established two potential mechanisms to explain the origin of luminal ATP in bile ducts, which are not mutually exclusive. One of the proposed pathways suggests that CFTR, either directly or indirectly, mediates the secretion of ATP from cholangiocytes to the lumen of the ducts[10, 11]. Once in the lumen ATP will act in autocrine/paracrine fashion to activate apical P2YR. A second proposed mechanism is that ATP is released via classical exocytosis of vesicles. In this scenario, ATP is co-released alongside the contents of exocytic vesicles and then activates the apical P2YR[14]. Third, there is also evidence that hepatocytes secrete ATP into bile, which is then detected by the cholangiocytes that are downstream[15, 16].

An additional aspect to be considered here is the morpho-functional distinction between small and large cholangiocytes. Small cholangiocytes are cuboidal epithelial cells which on average have a diameter ≤ 15 μm and line the lumen of intrahepatic bile ducts of 20 – 100 μm cross-section[17]. Functionally, these cholangiocytes do no express CFTR and their secretory activity is mainly driven by the ATP-Ca^{2+}-TMEM16A axis. Conversely, large cholangiocytes (diameter ≥ 15 μm) are columnar cells with a higher cytoplasm to nucleus ratio and form the walls of large intra-hepatic bile ducts (≥ 100 μm). Fluid secretion in this subtype of cholangiocytes is dependent on both Ca^{2+} and cAMP since both basolateral secretin receptors and apical CFTR are expressed in large cholangiocytes[18].

Water secretion via aquaporins (AQP) constitutes an additional component of the secretory activity of cholangiocytes. Aquaporins 1 and 4 (AQP1 and AQP4, respectively) are expressed in rat cholangiocytes and secretin activation of cAMP synthesis induces insertion of subapical vesicles containing AQP1 with the plasma membrane[19]. This increased expression of AQP1 on the apical surface of cholangiocytes contributes to the overall choleretic effect of secretin. AQP4 instead is mainly localized to the basolateral membrane and its localization does not change after secretin treatment. The expression profile of the 12 known AQPs is less well described in human and mouse cholangiocytes. A recent report identified mRNAs for all the known AQP isoforms in adult murine bile duct cells but the functional relevance of such findings has not been investigated[20]. Interestingly, the absence of AQP1 in mice has no impact on fluid secretion by cholangiocytes[21]. A general overview of the regulatory mechanisms of fluid secretion in cholangiocytes is presented in Figure 1.

InsP3 receptors in cholangiocytes

Ca^{2+} signaling in epithelial cells is dependent on both release from intracellular stores of the ER and Ca^{+2} influx through plasma membrane channels. Ca^{2+} release from the ER occurs via activation of members of two families of intracellular ER Ca^{2+} channels, the InsP3R and Ryanodine receptors (RyR)[22]. Each of these families has 3 known mammalian isoforms, which can be expressed in a variety of combinations and subcellular localizations in different cells types. Hepatocytes for example express the type I and type II InsP3R (InsP3R1 and InsP3R2, respectively), but not the type III InsP3R (InsP3R3)[23]. Full length RyRs are not present in hepatocytes, however a functional truncated form has been described[24]. InsP3R have been shown to modulate diverse cellular functions in hepatocytes ranging from cellular proliferation and energy metabolism to bile secretion[25–27].

Cholangiocytes in turn have all three InsP3R isoforms albeit at different levels of expression. InsP3R3 constitutes about 90% of the total InsP3R pool and InsP3R1 and InsP3R2 combined account for the remaining 10%[28]. Beyond being the predominant InsP3R isoform, InsP3R3 is also most concentrated along the subapical membrane space as opposed to the diffuse distribution of both InsP3R1 and InsP3R2[28]. Furthermore, this peri-apical concentration of InsP3R3 is seen in both small and large cholangiocytes (Figure 2). Silencing of each of the InsP3Rs in microdissected rat bile duct segments by siRNA demonstrated that InsP3R3, but not InsP3R1 and InsP3R2, is essential for both forskolin-mediate fluid secretion and alkalization of bile by cholangiocytes[10]. Although whole body

and tissue specific InsP3R3 knock out mice have been generated, no studies related to bile secretion or cholangiocytes function have been performed in these mice to date[29, 30].

Loss of InsP3R3 from cholangiocytes is a common event in a number of human cholestatic disorders as well as in animal models of cholestasis[2]. InsP3R3 was significantly reduced in cholangiocytes of liver samples from patients diagnosed with PSC, PBC, biliary atresia and secondary biliary obstruction. InsP3R3 is similarly reduced in three models of cholestasis in rats: 1) Two-week bile duct ligation (BDL); 2) acute lipopolysaccharide (LPS) treatment; 3) feeding with α-naphthylisothiocyanate (ANIT). These results provide evidence that InsP3R3 and its Ca^{2+} release properties are mechanistically linked to the pathogenesis of cholestasis.

Cholestasis in alcoholic liver disease (ALD)

ALD comprises a number of histological and clinical features ranging from hepatic steatosis to alcoholic hepatitis and liver cirrhosis, and is the result of excessive alcohol intake for prolonged periods of time[31]. Jaundice is commonly associated with alcoholic hepatitis (AH) and is thought to be caused primarily by hepatocellular damage. However, no clear cellular and molecular mechanism has been established as the underlying cause of jaundice in AH[32]. Recent observations that AH can include increased serum levels of alkaline phosphatase and histological evidence of cholestasis in liver biopsies, suggests that cholangiocyte damage and cholestasis may be present in AH as well, and may contribute to the jaundice seen in this disorder[3, 4]. Although studies employing rodent models of alcoholic liver injury have implicated a number of molecular mechanisms in the pathogenesis of alcoholic hepatitis including activation of tumor necrosis factor alpha (TNFα), formation of reactive oxygen species (ROS) and mitochondrial dysfunction and decreased hepatocyte regeneration, no significant recent advancement has been made in terms of targeted treatments of alcoholic liver diseases[32]. Our preliminary work in a small cohort of liver specimens from patients diagnosed with alcoholic hepatitis indicates that InsP3R3 is either reduced or mis-localized in bile ducts in the majority of patients (unpublished data). These results in association with the elevated serum alkaline phosphatase levels sometimes seen in AH patients raises the possibility that a dysfunction in secretory activity of cholangiocytes may contribute to cholestasis in AH and that InsP3R3 expression/activity mighty constitute an underlying molecular mechanism.

Regulation of InsP3R3 expression in cholestasis

Several mechanisms contribute to regulation of InsP3R expression and activity. Post-translational modifications such as serine phosphorylation by PKA and PKC were shown to modulate channel activity[33]. Protein interaction with both ER-lumen proteins (chromogranin-B) and cytoplasmic proteins (IRBIT and protein 4.1N) also regulate Ca^{2+} flow and InsP3R targeting[34–36]. Also InsP3R protein degradation by ubiquitination was shown to attenuate Ca^{2+} signaling in *in vitro* experiments[37]. However little is known regarding the transcriptional regulation of InsP3Rs, especially in terms of specificity for each of the three InsP3R isoforms. Studies in neurons revealed that InsP3R1 gene expression is regulated by TNF-α via specific protein 1 (SP1) binding elements on the InsP3R1 promoter. InsP3R2 expression was shown to be regulated by microRNA-133

(miR-133) in cardiomyocytes in the context of cardiac hypertrophy. Our group has recently reported that miR-506 mediates the downregulation of InsP3R3 expression in cholangiocyte cell lines[38]. This microRNA binds to seed sequences on the InsP3R3 mRNA 3′ untranslated region (UTR) and promotes the reduction of both InsP3R3 mRNA and protein expression. Additionally, treatment of isolated rat bile duct units with miR-506 mimics decreased forskolin-mediated fluid secretion. Moreover, increases in miR-506 correlated with reduced expression of InP3R3 in bile ducts of PBC patients. Together, these results suggest that miR-506 regulation of InsP3R3 expression might be relevant to the pathogenesis of specific forms of cholestatic diseases. Our preliminary work also indicates that additional mechanisms operate in the transcriptional regulation of InsP3R3 in bile ducts. One such mechanism is the down regulation of InsP3R3 expression by the nuclear factor erythroid 2-related factor 2 (Nrf2; data not shown). This transcription factor typically is activated by reactive oxygen species (ROS) and promotes the expression of a range of anti-oxidant enzymes[39]. Activated Nrf2 classically binds to a 41 base pair consensus sequence in promoter region of genes called ARE (anti oxidant response element)[40]. Work in progress is examining whether this regulates expression of InsP3R3 in cholangiocytes (unpublished data). Interestingly, Nrf2 was shown to be overexpressed in the liver after excessive alcohol use[41]. Together, these results show that multiple mechanisms are involved in the regulation of InsP3R expression and can be explored as potential mediators of cholestasis, including those that are associated with alcoholic hepatitis.

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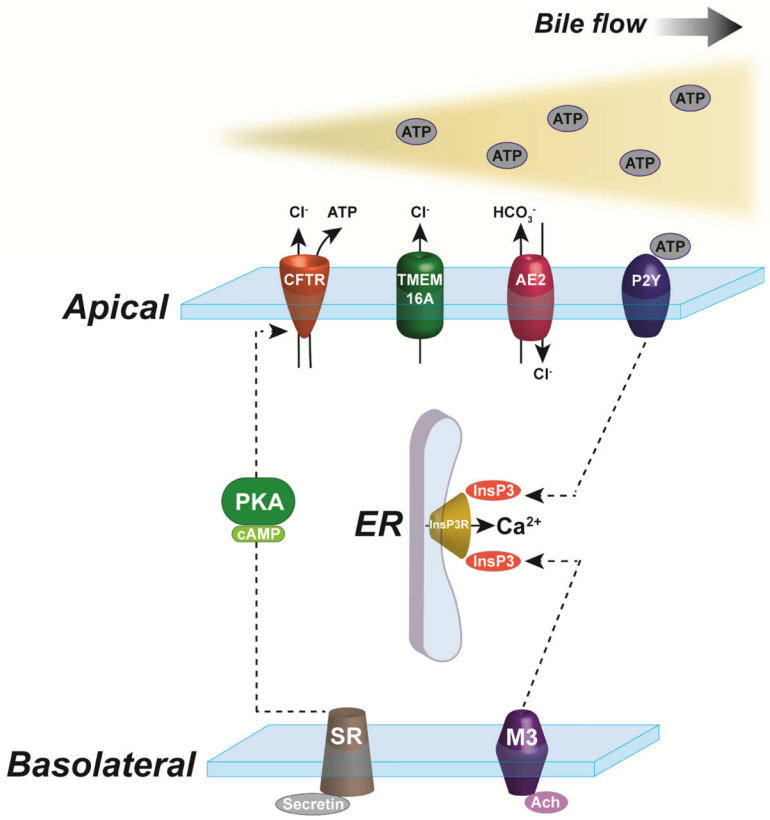

Figure 1. Regulatory mechanisms of fluid secretion in cholangiocytes

Activation of secretin receptors (SR) on the basolateral membrane leads to the formation of cAMP and PKA phosphorylation of CFTR, which promotes apical Cl^- efflux. Also originating at the basolateral domain, InsP3 formation due to M3 acetylcholine activation induces Ca^{2+} release from the ER, which activates Cl^- secretion through TMEM16A on the apical membrane. ATP, released by upstream hepatocytes or secreted by cholangiocytes in association with CFTR, binds apical P2Y receptors and stimulates further intracellular Ca^{2+} release. The Cl^- gradient across the apical membrane drives the AE2 exchanger, resulting in $HCO3^-$ secretion and alkalization of the bile.

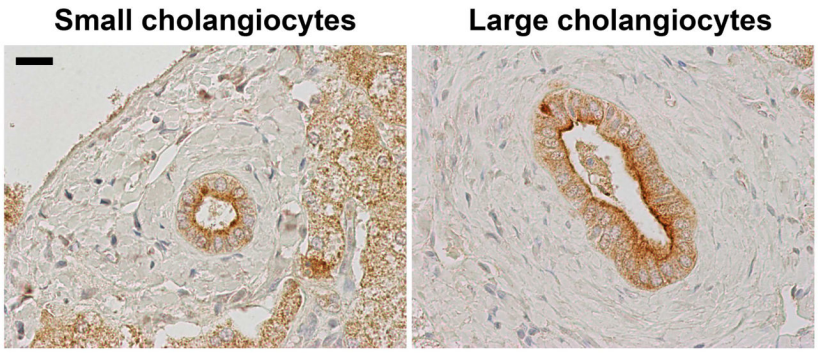

Figure 2. InsP3R3 is expressed in both small and large cholangiocytes

Immunohistochemistry staining of histologically normal human liver biopsies shows the presence of InsP3R3 (brown) concentrated near the apical membrane in both small (left) and large (right) cholangiocytes. Scale bar represents 20 μm.