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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, 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 phosphotidylinositol-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

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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 (Fig. 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 (Fig. 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].

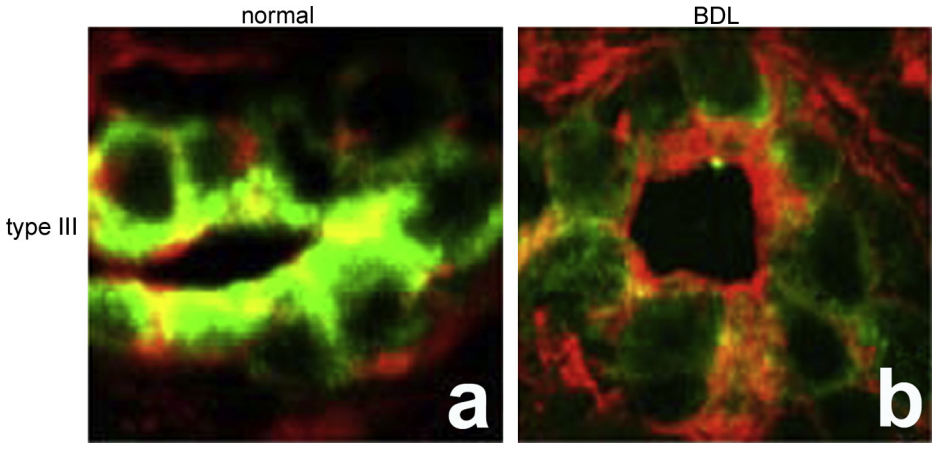

Fig. 1. Type III InsP3R is concentrated in the apical region of bile duct epithelia and is lost after bile duct ligation. Confocal immunofluorescence of liver sections from rats before and after bile duct ligation (BDL) and double-labeled with a type III-specific InsP3R antibody (green) and rhodamine 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.