

# Advances in $\text{Ca}^{2+}$ modulation of gastrointestinal anion secretion and its dysregulation in digestive disorders (Review)

WEIXI SHAN<sup>1\*</sup>, YANXIA HU<sup>1\*</sup>, JIANHONG DING<sup>1</sup>, XIAOXU YANG<sup>1</sup>, JUN LOU<sup>1</sup>,  
QIAN DU<sup>1</sup>, QIUSHI LIAO<sup>1</sup>, LIHONG LUO<sup>2</sup>, JINGYU XU<sup>1</sup> and RUI XIE<sup>1</sup>

<sup>1</sup>Department of Gastroenterology, The Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou 563003;

<sup>2</sup>Department of Oncology and Geriatrics, Traditional Chinese Medicine Hospital of Chishui City, Guizhou 564700, P.R. China

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**Abstract.** Intracellular calcium ( $\text{Ca}^{2+}$ ) is a critical cell signaling component in gastrointestinal (GI) physiology. Cytosolic calcium ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ), as a secondary messenger, controls GI epithelial fluid and ion transport, mucus and neuropeptide secretion, as well as synaptic transmission and motility. The key roles of  $\text{Ca}^{2+}$  signaling in other types of secretory cell (including those in the airways and salivary glands) are well known. However, its action in GI epithelial secretion and the underlying molecular mechanisms have remained to be fully elucidated. The present review focused on the role of  $[\text{Ca}^{2+}]_{\text{cyt}}$  in GI epithelial anion secretion.  $\text{Ca}^{2+}$  signaling regulates the activities of ion channels and transporters involved in GI epithelial ion and fluid transport, including  $\text{Cl}^-$  channels,  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels, cystic fibrosis (CF) transmembrane conductance regulator and anion/ $\text{HCO}_3^-$  exchangers. Previous studies by the current researchers have focused on this field over several years, providing solid evidence that  $\text{Ca}^{2+}$  signaling has an important role in the regulation of GI epithelial anion secretion and uncovering underlying molecular mechanisms. The present review is largely based on previous studies by the current researchers and provides an overview of the currently known molecular mechanisms of GI epithelial anion secretion with an emphasis on  $\text{Ca}^{2+}$ -mediated ion secretion and its dysregulation in GI disorders. In addition, previous studies by the current researchers demonstrated that different regulatory mechanisms are in place for GI epithelial  $\text{HCO}_3^-$  and  $\text{Cl}^-$  secretion. An increased understanding of the roles of  $\text{Ca}^{2+}$  signaling and its targets in GI anion secretion may lead

to the development of novel strategies to inhibit GI diseases, including the enhancement of fluid secretion in CF and protection of the GI mucosa in ulcer diseases.

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## 1. Introduction

It is well known that  $[\text{Ca}^{2+}]_{\text{cyt}}$  has an essential role in numerous important mammalian cell functions, including neurotransmitter release, gene regulation, muscle contraction, cell proliferation, differentiation and apoptosis (1). Since the function of  $[\text{Ca}^{2+}]_{\text{cyt}}$  as the cell's secondary messenger is based on the presence of a concentration gradient between  $[\text{Ca}^{2+}]_{\text{cyt}}$  and external  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_{\text{ext}}$ ) (2), normal  $[\text{Ca}^{2+}]_{\text{cyt}}$  homeostasis is important. Under normal physiological conditions, the concentration of  $[\text{Ca}^{2+}]_{\text{cyt}}$  is  $<100$  nM, which is  $\sim 10,000$  times lower than  $[\text{Ca}^{2+}]_{\text{ext}}$  ( $>1$  mM) (3,4). Furthermore, the membrane potential of  $\sim -60$  mV adds to the large electrochemical gradient and this huge concentration gradient favors the entry of  $\text{Ca}^{2+}$  into cells (5). Therefore, when cells are activated,  $\text{Ca}^{2+}$  is transported down this electrochemical gradient into the cells through the specific transmembrane  $\text{Ca}^{2+}$  channels, leading to increases in the  $[\text{Ca}^{2+}]_{\text{cyt}}$  (5).

To restore the resting levels of  $[\text{Ca}^{2+}]_{\text{cyt}}$ ,  $\text{Ca}^{2+}$  is transported back to the extracellular space or stored in intracellular  $\text{Ca}^{2+}$  pools by  $\text{Ca}^{2+}$  pumps and transporters (6,7). Furthermore,  $[\text{Ca}^{2+}]_{\text{cyt}}$  exhibits differences functions in different type of cells (8). These are key factors that determine different specific  $\text{Ca}^{2+}$ -dependent cellular responses affected by complex, spatiotemporal variations in  $[\text{Ca}^{2+}]_{\text{cyt}}$  (Fig. 1). A major determinant of these variations are different functionally distinct membrane calcium channels and exchangers, such as the receptor-operated calcium channels, voltage-gated channels,  $\text{Na}^+/\text{Ca}^{2+}$  exchangers (NCX) and calcium pumps (9). In addition, the intracellular stores are an important determinant

*Correspondence to:* Professor Jingyu Xu or Professor Rui Xie, Department of Gastroenterology, The Affiliated Hospital of Zunyi Medical University, 149 Dalian Road, Zunyi, Guizhou 563003, P.R. China  
E-mail: xujingyu\_gzzy@126.com  
E-mail: xr19841029@aliyun.com

\*Contributed equally

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for  $\text{Ca}^{2+}$  release (10). To date, the ryanodine receptor (RyR) and inositol triphosphate receptor ( $\text{IP}_3\text{R}$ ) channels on the endoplasmic reticulum (ER), have been identified, which lead to  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) or release of  $\text{IP}_3$ , respectively, to mediate the release of  $\text{Ca}^{2+}$  from intracellular stores and increasing the  $[\text{Ca}^{2+}]_{\text{cyt}}$  (11).

In the digestive system,  $[\text{Ca}^{2+}]_{\text{cyt}}$  also has critical roles in the regulation of digestive functions (12,13). This includes GI motility and ion transport, food digestion and nutrient absorption (14). In the epithelial cells of the GI tract, ion secretion and absorption of electrolytes and fluid are two essential functions and ion transport is also a critical physiological process in the human GI tract (15). GI epithelium secretes anions ( $\text{Cl}^-$  and  $\text{HCO}_3^-$ ), providing the driving force for fluid transport to maintain fluid homeostasis in the human body (16). GI epithelial anion secretion is controlled by several neuro-humoral factors, including prostaglandin E2 (PGE2), acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) (17). These factors mediate epithelial anion secretion mainly through  $\text{Ca}^{2+}$ , cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) signaling pathways (17).

The physiological roles and molecular mechanisms of cAMP- and cGMP-dependent regulation of GI epithelial anion secretion have been extensively defined (18). Since certain adenylyl cyclase subtypes are  $\text{Ca}^{2+}$ -dependent and multiple interactions between  $\text{Ca}^{2+}$  and cAMP signaling pathways exist in mammalian cells, it was previously thought that  $\text{Ca}^{2+}$  may mediate epithelial anion secretion indirectly through cAMP signaling (19).

However, various lines of evidence indicate that  $\text{Ca}^{2+}$  signaling is able to mediate epithelial anion secretion in a cAMP-independent manner (19,20). Although the critical role of  $\text{Ca}^{2+}$ -dependent regulation has been verified, the underlying regulatory mechanisms remain to be fully elucidated. The current researchers have been investigating the  $\text{Ca}^{2+}$ -mediated regulation of anion secretion by GI epithelium and provided solid evidence for a regulatory role of  $\text{Ca}^{2+}$  signaling in a cAMP-independent manner, as well as the underlying molecular mechanisms (19).

While the critical role of  $\text{Ca}^{2+}$  signaling in other types of secretory cell (such as those of the airways and salivary gland) is well known (21), the roles of  $\text{Ca}^{2+}$  signaling in GI epithelial secretion and its molecular mechanisms have remained to be fully elucidated. Further research is required to molecularly identify the  $\text{Ca}^{2+}$ -activated ion transporters in GI epithelial cells. In addition, the application of muscarinic agonists was observed to lead to an increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$  in secretory cells and promote GI anion secretion (22). However, this phenomenon has been extensively studied in other types secretory cells, such as the secretory cells in the avian basal gland (21).

Overall, the  $\text{Ca}^{2+}$  signaling and anion secretion mechanisms are of important clinical relevance and need to be further elucidated. In cystic fibrosis (CF), certain calcium agonists stimulate  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channel (CaCC)-dependent and CF transmembrane conductance regulator (CFTR)-independent secretion and agents that stimulate  $\text{Ca}^{2+}$  signaling may be used therapeutically to restore ion and fluid secretion defects (23). Conversely, in certain other types of GI disease, such as intestinal inflammation and diarrhea, inhibition of intracellular  $\text{Ca}^{2+}$  signaling may have therapeutic potential to ameliorate

excessive fluid secretion (24,25) (Table I). Therefore, in the present review, the current state of knowledge regarding  $\text{Ca}^{2+}$  signaling in the regulation of GI epithelial anion secretion and the associated GI disorders was summarized.

## 2. General aspects of GI epithelial anion secretion

**GI epithelial  $\text{HCO}_3^-$  secretion.** GI epithelial bicarbonate ( $\text{HCO}_3^-$ ) is produced on the surface GI epithelial cells and secreted to the luminal side of the epithelium; it is involved in the formation of gastrointestinal mucus with a slightly alkaline pH (12). The basolateral side of electroneutral  $\text{Na}^+$ -coupled  $\text{HCO}_3^-$  cotransporter is one of the important transporters for  $\text{HCO}_3^-$  absorption (12). With the help of the carbonic anhydrase, the  $\text{HCO}_3^-$  is taken up and it is also generated inside the cells (26).

To date, several pathways for the export of  $\text{HCO}_3^-$  into the luminal side of the GI mucosa have been elucidated: i) The CFTR or the CaCC is able to promote electrogenic  $\text{HCO}_3^-$  efflux, ii) luminal electroneutral anion/ $\text{HCO}_3^-$  exchangers have been confirmed to contribute to the transport of  $\text{HCO}_3^-$  and iii)  $\text{HCO}_3^-$  may be transported via the short-chain fatty acids (SCFA)/ $\text{HCO}_3^-$  exchanger in the colon (27,28). Electroneutral secretion of  $\text{HCO}_3^-$  is paralleled by the activity of  $\text{Na}^+/\text{H}^+$  exchanger-3 (29). Furthermore, the luminal  $\text{Cl}^-$  channels, as a recycling pathway for  $\text{Cl}^-$ , are important for  $\text{HCO}_3^-$  secretion and they may serve in the electrogenic secretion of  $\text{HCO}_3^-$  via the luminal  $\text{Cl}^-/\text{HCO}_3^-$  antiporters (27). One of the  $\text{Cl}^-$  channels, CFTR, which is located on the apical side, was clearly demonstrated to be involved in the response of  $\text{HCO}_3^-$  secretion in the intestine a pancreatic duct (30). Besides the CFTR,  $\text{Ca}^{2+}$ , cAMP and cGMP were indicated to induce  $\text{HCO}_3^-$  secretion in the small intestine (31). Current data has also demonstrated that the CFTR is involved in the regulation of the intracellular pH and that the expression and function of  $\text{Cl}^-/\text{HCO}_3^-$  exchangers and down-regulated in adenoma were regulated by the CFTR (32). Of note, CFTR is necessary for  $\text{HCO}_3^-$  secretion in numerous other epithelial tissues as well (33). Thus, it is likely that the CFTR contributes to anion secretion and control of the luminal pH in the entire GI tract, but in the mammalian colon, the SCFA-dependent  $\text{HCO}_3^-$  secretion is the primary mechanism of  $\text{HCO}_3^-$  secretion (34). Well-regulated  $\text{HCO}_3^-$  secretion is critical for the mucosal defense against luminal acid due to its neutralization effect in the upper GI tract and against bacteria in the lower GI tract due to its stimulation of mucus secretion and maintenance of the intestinal barrier function (35). Aside from mechanisms of mucosal protection, normal  $\text{HCO}_3^-$  secretion in the small intestine is assumed to establish and maintain an optimal pH for the activity of luminal digestive enzymes (35). The small intestine is in an alkaline range of pH 6.7-8.0, which is the best pH value for the optimal activity of pancreatic enzymes (36). The duodenum in particular is an important organ exerting pH control for enzymatic digestion (37).

Defective intestinal  $\text{HCO}_3^-$  secretion has been indicated to be a risk factor for intestinal inflammation and peptic ulcer diseases (38). Furthermore, intestinal  $\text{HCO}_3^-$  secretion has been critically involved in the pathophysiology of acute infectious diarrhea (39). Cholera and numerous other acute diarrheal illnesses may increase the intestinal secretion and

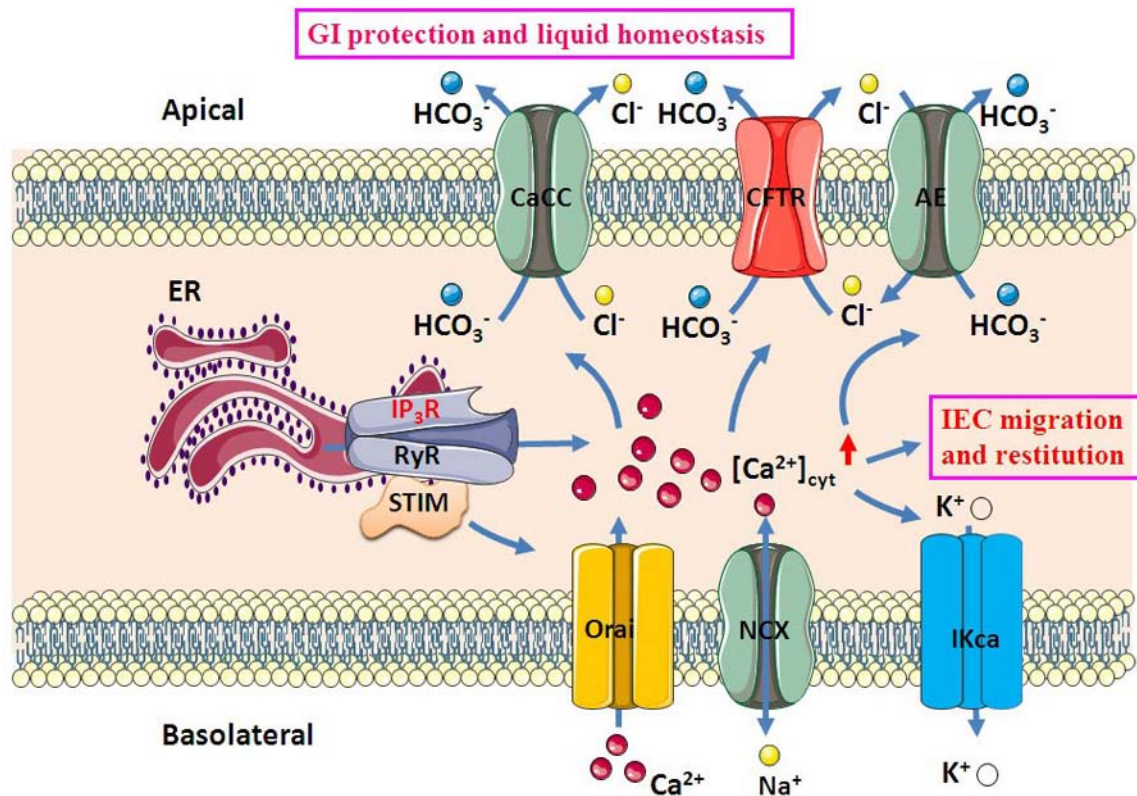


Figure 1.  $\text{Ca}^{2+}$ -mediated GI epithelial anion secretion. An increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$  resulted from intracellular  $\text{Ca}^{2+}$  release through  $\text{IP}_3\text{R}$  or  $\text{RyR}$  on the ER and extracellular  $\text{Ca}^{2+}$  entry through  $\text{Orai}$  and  $\text{NCX}$  on the plasma membrane stimulates apical  $\text{CaCC}$ ,  $\text{AE}$  and  $\text{CFTR}$ , as well as basolateral  $\text{IKCa}$ .  $\text{Ca}^{2+}$  signaling activates IEC migration and restitution and stimulates  $\text{HCO}_3^-$  and  $\text{Cl}^-$  secretion, which induces epithelial protection and liquid homeostasis, respectively.  $\text{CaCC}$ ,  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channel;  $\text{AE}$ , anion exchange;  $\text{CFTR}$ , cystic fibrosis transmembrane conductance regulator;  $\text{IKCa}$ , intermediate-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channel;  $\text{ER}$ , endoplasmic reticulum;  $\text{RyR}$ , ryanodine receptor;  $\text{IP}_3\text{R}$ , inositol triphosphate receptor;  $\text{STIM}$ , stromal interaction molecule;  $\text{Orai}$ ,  $\text{ORAI}$  calcium release-activated calcium modulators;  $\text{NCX}$ ,  $\text{Na}^+/\text{Ca}^{2+}$  exchangers; IEC, intestinal epithelial cell; GI, gastrointestinal.

loss of  $\text{HCO}_3^-$ , which may result in a severe  $\text{HCO}_3^-$  deficit and metabolic acidosis (40). Defective GI epithelial  $\text{HCO}_3^-$  secretion has been critically implicated in the pathogenesis of CF (30). A previous study examining the human duodenum indicated a  $\text{CFTR}$ -dependent alkaline transport in subjects without CF, which was absent in patients with CF (41). Furthermore, electrogenic  $\text{HCO}_3^-$  secretion was detected in the colon of mice without CF, while it was absent in mice with CF (42). The defective  $\text{HCO}_3^-$  transport in CF may be crucial for the severity of the symptoms of CF (43). Defective  $\text{HCO}_3^-$  transport probably causes obstruction of the pancreatic duct and exocrine pancreatic insufficiency (44). Impaired duodenal  $\text{HCO}_3^-$  production and failure to buffer gastric acid is responsible for an increased incidence of epigastric pain and morphological changes in the duodenum of patients with CF (45).

**GI epithelial  $\text{Cl}^-$  secretion.** In GI physiology, fluid secretion has a critical role and is driven by active  $\text{Cl}^-$  transport from the basolateral to the apical side of enterocytes (46). The basolateral  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  cotransporter ( $\text{NKCC1}$ ) is one of the important transporters for  $\text{Cl}^-$  secretion (47). The rate secretion of  $\text{Cl}^-$  is regulated by the activity of  $\text{NKCC1}$ , which is dependent on the intracellular  $\text{Cl}^-$  concentration, cell swelling and probably phosphorylation (47). It has also been confirmed that the cAMP-activated  $\text{KVLQT1/KCNE3}$  and  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels are able to maintain  $\text{Cl}^-$  transport (48). The basolateral

$\text{Cl}^-$  is taken up by  $\text{NKCC}$ ; however, its exit is primarily via the apical  $\text{CFTR}$ . Channels such as  $\text{CaCC}$  and other  $\text{Cl}^-$  channels may also take part in apical  $\text{Cl}^-$  secretion (49).  $\text{Na}^+$  and water follow via a paracellular route (50). These ion and fluid transports initiated by pathogens (e.g., cholera toxin and rotavirus) involve multiple factors, such as 5-HT, substance P, ACh and vasoactive intestinal peptide, as well as the release of inflammatory mediators from mast cells and neutrophils [e.g. interleukins (ILs) and prostaglandins] (51). Ion and fluid secretion may be activated by different mechanisms that involve second messengers (cAMP, cGMP or  $\text{Ca}^{2+}$ ) to activate membrane ion channels (20).

**Differences between GI epithelial secretion of  $\text{Cl}^-$  and  $\text{HCO}_3^-$ .** It is generally assumed that the GI epithelial  $\text{HCO}_3^-$  and  $\text{Cl}^-$  secretion have the same regulatory mechanisms (52). However, this notion requires to be confirmed through a systematic comparison between them (52). As mentioned earlier, GI epithelial  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretion is mainly controlled by cAMP and  $\text{Ca}^{2+}$  signaling, which may interact and cross-talk to regulate epithelial ion transport (13,27). Previous studies have demonstrated that most well-known secretagogues, including 5-HT, ACh, forskolin and  $\text{PGE}_2$ , stimulate intestinal  $\text{HCO}_3^-$  and  $\text{Cl}^-$  secretion in parallel (53-55). However, whether epithelial  $\text{HCO}_3^-$  and  $\text{Cl}^-$  secretion occur in parallel and whether they are regulated by the same or different signaling/mechanisms currently

Table I. Ca<sup>2+</sup>-mediated GI epithelial anion secretion and the membrane ion channels involved.

Ion channel	Mechanism	Expression	Related diseases	Author (refs)
HCO <sub>3</sub> <sup>-</sup>	Maintenance of intestinal barrier function. Mechanisms of mucosal protection. Establishes and maintains optimal pH for the activity of luminal digestive enzymes	GI epithelial cells	Intestinal inflammation, peptic ulcers, acute infectious diarrhea, metabolic acidosis, CF, obstruction of the pancreatic duct, exocrine pancreatic insufficiency, CLD, IBD	Chen <i>et al</i> (26) Fei <i>et al</i> (36) Kuna <i>et al</i> (38) Gennari <i>et al</i> (40) Ramos <i>et al</i> (45)
Cl <sup>-</sup>	Transports the secreted fluid from the lateral base to the apical side of intestinal cells	Basolateral GI epithelial cells	Acute infectious diarrhea, metabolic acidosis, CLD, IBD	Frizzell <i>et al</i> (27) Mohammad-Panah <i>et al</i> (49)
CFTR	Contributes to the secretion of anions and fluids in enterotoxin-induced secretory diarrhea	Pancreas, epithelial cells in airways, intestinal tract	CF	Goodman <i>et al</i> (66) Deachapunya <i>et al</i> (67)
CaCC	May participate in anion secretion in the mammalian GI epithelium	Intestinal tract	CF	Caputo <i>et al</i> (76) Yang <i>et al</i> (17) Morris <i>et al</i> (79) Kunzelmann <i>et al</i> (83)
Anion/HCO <sub>3</sub> <sup>-</sup> exchangers	Protects gastric mucosa by combating aggressive factors	Apical membrane of intestinal epithelial cells	Acute infectious diarrhea, metabolic acidosis, IBD, CLD, CF	Singh <i>et al</i> (92) Tang <i>et al</i> (59) Smith <i>et al</i> (94)
SOC and STIM/Orai channels	Gene expression, cell growth and organ development	Intestinal epithelial cells	CF	Rao <i>et al</i> (105) Onodera <i>et al</i> (106)
K <sub>Ca</sub>	Contributes to the stabilization of membrane voltage and provides the driving force for electrogenic anion transport	Intestinal epithelium	CLD, IBD, secretory diarrhea, CF	Julio-Kalajzić <i>et al</i> (114) Dong <i>et al</i> (121,127) Xie <i>et al</i> (13) Assaha <i>et al</i> (115) Wang <i>et al</i> (116)
NCX	Maintenance of Ca <sup>2+</sup> homeostasis in a variety of tissues. Involved in the Ca <sup>2+</sup> -dependent anion secretion	Cardiomyocytes, vascular cells, neurons, small intestinal epithelial cells	Intestinal inflammation, acute infectious diarrhea, metabolic acidosis, CF, CLD, IBD	Lee <i>et al</i> (124) Seipet <i>et al</i> (126) Dong <i>et al</i> (121,127) Kocks <i>et al</i> (110)

GI, gastrointestinal; HCO<sub>3</sub><sup>-</sup>, bicarbonate; CF, cystic fibrosis; CLD, congenital chloride diarrhea; IBD, inflammatory bowel disease; Cl<sup>-</sup>, chloride; CFTR, cystic fibrosis transmembrane conductance regulator; CaCC, Ca<sup>2+</sup>-activated Cl<sup>-</sup> channel; SOC, store-operated calcium channels; STIM, stromal interaction molecule; Orai, ORAI calcium release-activated calcium modulators; K<sub>Ca</sub>, Ca<sup>2+</sup>-activated K<sup>+</sup> channel; NCX, Na<sup>+</sup>/Ca<sup>2+</sup> exchangers.

remains elusive. Notably, it has been indicated that both forskolin- and carbachol (CCh)-induced rat colonic Cl<sup>-</sup> secretion was inhibited by estrogen (56) and further studies by the current researchers revealed that estrogen stimulates duodenal bicarbonate secretion (DBS) in humans and mice without altering basal duodenal short-circuit current (*I*<sub>sc</sub>), an index primarily of epithelial Cl<sup>-</sup> secretion (57,58). These results demonstrated that estrogen may have different roles in regulating intestinal HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> secretion. These findings also suggest that GI epithelial HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> secretion may not be necessarily triggered in the same way or by identical signaling/mechanisms. Furthermore, a previous study

by the current researchers revealed that calcium-sensing receptor (CaSR) activation raises [Ca<sup>2+</sup>]<sub>cyt</sub>; however, it reduces cAMP-induced exclusive duodenal HCO<sub>3</sub><sup>-</sup> secretion without simultaneously altering duodenal Cl<sup>-</sup> secretion (13). Similarly, Tang *et al* (59) demonstrated that CaSR activation stimulated colonic HCO<sub>3</sub><sup>-</sup> secretion via SCFA/HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchangers; however, it inhibited Cl<sup>-</sup> secretion via the cAMP/CFTR pathway. It may, therefore, be proposed that a different regulatory mechanism likely exists for GI epithelial HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> secretion. While cAMP may have a critical role in CFTR-mediated Cl<sup>-</sup> secretion, Ca<sup>2+</sup> signaling may be critical in anion/HCO<sub>3</sub><sup>-</sup>-mediated HCO<sub>3</sub><sup>-</sup> secretion.



### 3. $\text{Ca}^{2+}$ modulation of GI epithelial anion secretion

**Evidence for  $\text{Ca}^{2+}$ -mediated anion secretion.** Although  $\text{Ca}^{2+}$  may mediate epithelial anion secretion through the cAMP signaling pathway, growing lines of evidence indicate that  $\text{Ca}^{2+}$  signaling is able to mediate epithelial anion secretion in a cAMP-independent manner (19). The evidence is as follows: i) The increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$  induced by stimulation of cholinergic muscarinic type 3 receptor (M3R) were indicated to be due to activation of basolateral  $\text{K}^+$  channels, which enhanced the driving force for luminal anion exit (60); ii)  $\text{Ca}^{2+}$ /calmodulin and protein kinase C (PKC) was demonstrated to be involved in the CCh-mediated regulation of luminal and basolateral  $\text{K}^+$  channels (61); iii) several previous studies suggested a contribution of  $\text{Ca}^{2+}$ /PKC to CFTR activation (62,63); and iv) activation of muscarinic receptors resulted in an increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$ ; however, it decreased cAMP levels, which indeed triggered  $\text{Ca}^{2+}$ -dependent duodenal transepithelial  $\text{HCO}_3^-$  secretion (13). Since cAMP-mediated ion transport has been extensively reviewed (64), the present study focused on  $\text{Ca}^{2+}$ -mediated GI epithelial anion secretion and the membrane ion channels involved.

**Apical CFTR.** CFTR is expressed in different tissue types, including the pancreas, epithelial cells in the airways, GI tract and other fluid-transporting tissues (30). CF is caused by mutations in the CFTR gene, resulting in impaired  $\text{Cl}^-$  and  $\text{HCO}_3^-$  transport and plasma membrane targeting (65). CFTR is mainly located in the luminal membrane of enterocytes and has a major role to contribute to the secretion of anions and fluid in enterotoxin-induced secretory diarrheas such as cholera (66). Numerous lines of solid evidence suggest a pivotal role for CFTR in GI anion and fluid secretion (27,30,65,66).

Numerous *in vitro* studies have indicated that the application of glibenclamide and 5-nitro-2-(3-phenylpropylamino) benzoic acid further inhibited the  $\text{PGE}_2$  and cAMP-mediated increase in  $I_{\text{sc}}$  and anion secretion in GI epithelial sheets and cell lines (67,68). Mice with gene ablation of CFTR developed intestinal obstruction (69,70). The resultant characteristic ion transport impairment resulted in defective intestinal anion and fluid secretion and increased fluid absorption (71). In CFTR-null mice, increased expression of alternative  $\text{Cl}^-$  channels was present and the development of mild intestinal symptoms was observed (72). Furthermore, in CFTR-knockout mice, cholera toxin failed to cause massive fluid secretion through CFTR-dependent protein (72). CFTR has long been considered a primarily cAMP-activated  $\text{Cl}^-$  channel to activate GI epithelial anion secretion (30). The majority of studies have confirmed that CFTR also responds to  $\text{Ca}^{2+}$ -mobilizing secretagogues and contributes substantially to cholinergic and purinergic responses in native tissues (30,62).

CFTR channels may be stimulated by the G protein-coupled receptor-mediated signal via Gq protein  $\alpha$  subunit, further activating  $\text{Ca}^{2+}$ -dependent adenylyl cyclase and tyrosine kinases, and by inhibition of protein phosphatase type 2A (PP2A) (72). For instance, the M3R couples strongly to  $\text{G}_{\alpha_q}$ . Stimulation of M3R produces diacylglycerol and  $\text{IP}_3$  to activate PKC and mobilize intracellular  $\text{Ca}^{2+}$ , which in turn activates the proline-rich tyrosine kinase 2/Src complex (62). Src stimulates CFTR activity by phosphorylating it directly and inhibiting

its dephosphorylation through the inactivation of PP2A (62). Under basal conditions, constitutive  $\text{Ca}^{2+}$  entry through store-operated  $\text{Ca}^{2+}$  channels partially activates adenylyl cyclase and induces tonic CFTR activity (62).

A recent *in vitro* study by the current researchers revealed that the stimulation of mouse duodenal  $I_{\text{sc}}$  by CCh was significantly inhibited in a  $\text{Ca}^{2+}$ -free solution (17). After the application of CCh, the intracellular calcium was significantly increased; however, there was no increase cAMP and compared to the CFTR-knockout mice. CCh-induced  $\text{Ca}^{2+}$  was involved in the duodenal  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretion in wild-type mice. The CCh-induced intracellular calcium signaling also stimulated the phosphorylation of CFTR and promoted the CFTR transport to the plasma membrane of duodenal epithelial cells. Furthermore, CCh induced duodenal ion secretion and stimulated PI3K/Akt signaling pathway in duodenal epithelium and all of these effects were attenuated by selective PI3K inhibitors. Therefore, a novel molecular mechanism of  $\text{Ca}^{2+}$  signaling in CFTR-mediated ion secretion via PI3K/Akt was indicated.

Rasmussen *et al* (73), revealed that cigarette smoking increased  $[\text{Ca}^{2+}]_{\text{cyt}}$ -induced CFTR internalization, which was prevented by chelation of cytoplasmic  $\text{Ca}^{2+}$ . Furthermore, this phenomenon was inhibited by the macrolide antibiotic bafilomycin A1, which inhibited cigarette smoking-induced  $\text{Ca}^{2+}$  release and prevented CFTR clearance from the plasma membrane, further linking cytoplasmic  $\text{Ca}^{2+}$  and CFTR internalization. Patel *et al* (74), also indicated that an increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$  induced a reduction of cell surface CFTR expression. Therefore, CFTR appears to be the channel that is in charge of not cAMP-activated,  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretion in human GI mucosa (30).

**Apical CaCC.** There is currently evidence that the CaCC are a further class of important  $\text{Cl}^-$  channels that may participate in anion secretion in the mammalian GI epithelium (75). In luminal membranes of GI epithelia of subjects with and without CF, CaCC are stimulated by  $\text{Ca}^{2+}$  ionophores and  $\text{Ca}^{2+}$ -mobilizing secretagogues (76), including acetylcholine, bradykinin, histamine, CCh and extracellular nucleotides adenosine triphosphate (ATP) and uridine triphosphate (UTP) (77,78). In mice with CF, the expression of  $\text{Ca}^{2+}$ -dependent  $\text{Cl}^-$  channels was detectable in the intestine and was age-dependent. In young mice (age, 2-3 weeks),  $\text{Cl}^-$  secretion was induced by carbachol in the small intestine (17). Furthermore, it was indicated that in non-CF and CF mouse pup crypts, the application of nonstructural protein 4 (NSP4) caused severe diarrhea (79). However, compared to the young CF mice, the NSP4-induced  $\text{Cl}^-$  secretion was largely reduced in adult CF mice. These data further support that the expression and function of CaCC are age-dependent. Indeed, it was also revealed that the adult CF mice (age, 6-12 weeks) did not exhibit CFTR-dependent  $\text{Cl}^-$  secretion; however, they did have a partial CFTR-independent duodenal  $\text{HCO}_3^-$  secretion in response to CCh (17). Therefore, CaCC may have an important role in the regulation of intestinal  $\text{Cl}^-$  secretion in young CF mice and may be important for duodenal  $\text{HCO}_3^-$  secretion in adult CF mice.

The  $\text{Ca}^{2+}$ -activated TMEM16A anion channel (or anoctamin 1) was reported to be able to conduct  $\text{HCO}_3^-$  upon a

significant increase in cytosolic  $\text{Ca}^{2+}$  levels (80). However, the role of anoctamin 1 in GI epithelial anion secretion remains under debate (81). More recently, a study by the current researchers indicated that caffeine-stimulated  $\text{Ca}^{2+}$ -dependent duodenal anion secretion was attenuated by niflumic acid and T16Ainh-A01, two selective CaCC blockers with different chemical structures, suggesting that the TMEM16A anion channel is likely one of the downstream effectors of  $\text{Ca}^{2+}$  signaling (82).

It has been demonstrated that a residual cholinergic  $\text{Cl}^-$  secretion was preserved in a subset of patients with CF with a mild phenotype (83). In T84 colonic carcinoma cells, the role of CaCC has also been characterized and it was indicated to be responsible for  $\text{Ca}^{2+}$ -mediated  $\text{Cl}^-$  secretion in these cells (83). However, other studies suggested that the integrated function of CFTR is important for CaCC (84), as  $\text{Ca}^{2+}$ -dependent cholinergic  $\text{Cl}^-$  secretion was able to be completely inhibited by the deactivation of CFTR (85). All those results suggest that residual cholinergic  $\text{Cl}^-$  secretion in CF tissues depends on the residual function of mutant CFTR. Therefore, although there is evidence for an alternative CaCC in the mouse colon and human colonic carcinoma cell lines, the promotion by CaCC is probably limited (86).

**Apical anion/ $\text{HCO}_3^-$  exchangers.** It has been generally accepted that  $\text{HCO}_3^-$  secretion from the upper GI tract is important for the protection of normal mucosa (87). There are three anion/ $\text{HCO}_3^-$  exchangers: Solute carrier family 26 member 6 [SLC26A6; also known as putative anion transporter 1 (PAT1)], DAR and SLC4A9 (also known as anion exchange protein 4); all those channels contribute to the DBS to resist various aggressive factors, such as the acidic gastric output (88,89). However, at least three distinct mechanisms of  $\text{HCO}_3^-$  secretion have been described in the distal colon of rats (90,91): i)  $\text{Cl}^-$ -dependent: The  $\text{HCO}_3^-$  secretion mediated by a brush-border  $\text{Cl}^-/\text{HCO}_3^-$  exchange; ii) SCFA-dependent:  $\text{HCO}_3^-$  secretion as a result of activation of SCFA/ $\text{HCO}_3^-$  exchange; and iii) cAMP-induced:  $\text{HCO}_3^-$  secretion associated with a CFTR (91).

As  $\text{Cl}^-/\text{HCO}_3^-$  exchanger was expressed on the apical membrane of the small intestinal epithelium and likely has a role in secretagogue-stimulated DBS (92,93), its possible involvement in estrogen-stimulated DBS was assessed in a study by the current researchers (94). The results suggested that estradiol ( $\text{E}_2$ ) indeed stimulated murine DBS, which was attenuated by 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid, a commonly used inhibitor of  $\text{Cl}^-/\text{HCO}_3^-$  exchanger.  $\text{E}_2$  was also able to increase  $[\text{Ca}^{2+}]_{\text{cyt}}$  in duodenal epithelial cells expressing estrogen receptor, whereas 1,2-Bis(2-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid tetrakis (acetoxymethyl ester) (BAPTA-AM) one of an intracellular calcium chelator inhibited the  $\text{E}_2$ -stimulated murine DBS. It was therefore suggested that the activation of estrogen receptor stimulates the  $\text{Ca}^{2+}$ -dependent DBS via  $\text{Cl}^-/\text{HCO}_3^-$  exchanger.

Colonic bicarbonate secretion (CBS) is closely linked to electrolyte movement and overall fluid in the colon (59). As mentioned earlier, CaSR activation increases  $[\text{Ca}^{2+}]_{\text{cyt}}$ ; however, it decreases intracellular cAMP production. Consistently, Tang *et al* (59) reported that CaSR activation inhibited cAMP-activated CBS; however, it increased the lumen  $\text{Cl}^-$ - and SCFA-dependent CBS. Consequently, upon activation

of electroneutral  $\text{Cl}^-/\text{HCO}_3^-$  and SCFA/ $\text{HCO}_3^-$  exchangers, CaSR stimulated CBS; by contrast, if forskolin-stimulated electrogenic CFTR-mediated  $\text{HCO}_3^-$  conductance dominated, CaSR inhibited CBS. Consistently with the results on the  $\text{Ca}^{2+}$ -mediated regulation of  $\text{Cl}^-/\text{HCO}_3^-$  exchanger-mediated DBS reported by the current researchers (13), these results further suggest a critical role of  $\text{Ca}^{2+}$  signaling in the regulation of  $\text{Cl}^-/\text{HCO}_3^-$  and SCFA/ $\text{HCO}_3^-$  exchanger-mediated CBS (59). Therefore, modulation of CaSR activity may provide a new therapeutic approach to correct  $\text{HCO}_3^-$  deficits and metabolic acidosis, a primary cause of morbidity and mortality in acute infectious diarrheal illnesses (59). However, Lamprecht *et al* (95) reported on  $\text{Ca}^{2+}$ -mediated inhibition of colonic DRA. Both the calcium ionophore A23187 and UTP that increased  $\text{Ca}^{2+}$  were able to inhibit DRA in these cells.

Basolateral store-operated channels and stromal interaction molecule (STIM)/Orai calcium release-activated calcium modulators. Store-operated calcium channels (SOC) are a major pathway for calcium signaling in virtually all mammalian cells and involved a variety of functions, including gene expression, cell growth and organ development (96-98). The SOC is stimulated by the diverse set of surface receptors via depletion of the  $\text{Ca}^{2+}$  concentration from the ER (99). The stromal interaction molecule (STIM) proteins were identified as the ER  $\text{Ca}^{2+}$  sensors and the Orai proteins as store-operated channels and since then, rapid progress has been made in the elucidation of the unique mechanisms of store-operated calcium entry (SOCE) (99). The role of STIM1/Orai signaling was previously studied mostly in nonpolarized cells, such as lymphocytes (100,101) or nonconfluent 293 cells (102,103). However, only a few studies have assessed the function of STIM1/Orai signaling in polarized GI epithelia (104-106). In the human colonic tumor cell line NCM460, STIM1 stimulated by the emptying of intracellular  $\text{Ca}^{2+}$  stores after the production of cAMP (104). In addition, in the intestinal epithelial cell (IEC)-6 cell line from rat intestinal crypts, STIM1/Orai was indicated to have a role in wound healing (105). In the rat colonic epithelium, STIM1/Orai was identified as a key component of intracellular  $\text{Ca}^{2+}$  signaling involved in the regulation of both apical and basolateral  $\text{Ca}^{2+}$  influx (106).

Although the critical role of  $\text{Ca}^{2+}$  signaling in GI epithelial anion secretion is well-known (13), the mechanisms by which  $[\text{Ca}^{2+}]_{\text{cyt}}$  homeostasis in GI epithelial cells is controlled remain to be fully elucidated (11). Under normal physiological conditions, in non-excitable epithelial cells, the occurrence of  $\text{Ca}^{2+}$  entry mainly depends on the SOC (99). In non-excitable cells, agonists induce  $\text{Ca}^{2+}$  signaling mostly depending on the intracellular  $\text{Ca}^{2+}$  release mainly from the ER and  $\text{Ca}^{2+}$  influx from the extracellular medium (107).  $\text{IP}_3$ -sensitive and ryanodine-sensitive  $\text{Ca}^{2+}$  stores have been identified within the ER (108,109). The former is activated by the binding of  $\text{IP}_3$  to  $\text{IP}_3\text{R}$ , while the latter is activated by the binding of ryanodine to RyR to induce ER  $\text{Ca}^{2+}$  release into the cytosol (110).

The  $\text{IP}_3\text{R}$ -mediated  $\text{Ca}^{2+}$  influx pathway was reported to have a role in the regulation of GI epithelial anion secretion (109,111). Similarly, a study by the current researchers indicated that muscarinic receptors were activated after the application of CCh and induced mouse intestinal  $\text{Cl}^-$  secretion, which was significantly inhibited by selective SOC blockers added to the serosal side of duodenal tissues in a  $\text{Ca}^{2+}$ -free

serosal solution (82). Furthermore, the study revealed that calcium release-activated calcium/Orai channels may represent the molecular candidate of SOC involved in the CCh-induced increase of intracellular  $\text{Ca}^{2+}$  in GI epithelium. As the underlying mechanisms of RyR-mediated ER  $\text{Ca}^{2+}$  release as an important component of SOCE to contribute to GI epithelial anion secretion had remained elusive, the role of RyR/ $\text{Ca}^{2+}$  storage was also further investigated by Dong *et al* (82). The results suggested that caffeine, a selective RyR activator, markedly increased mouse intestinal  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretion. However, this process was suppressed by  $\text{Ca}^{2+}$ -free serosal solutions and selective blockers of SOC/ $\text{Ca}^{2+}$  and knockdown of the protein expression of Orai1 channels also inhibited the  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretion on the serosal side of duodenal tissue. Furthermore, the caffeine-induced anion secretion was inhibited by ER  $\text{Ca}^{2+}$  chelator and RyR blockers (82). In addition, the protein expression of STIM1 and Orai1 was detected. In IEC cells, the caffeine-induced SOCE was attenuated by selective SOC inhibitor (82).

It was therefore concluded that the RyR/Orai1/ $\text{Ca}^{2+}$  signaling on the basolateral side has a critical role in the regulation of GI epithelial anion secretion (67). Lefkimmatis *et al* (112) indicated that in a newly identified type of SOC termed 'store-operated cAMP signaling' (SOcAMPS), the luminal ER  $\text{Ca}^{2+}$  sensor STIM1 does not depend on changes in  $[\text{Ca}^{2+}]_{\text{cyt}}$ . The decreasing free  $\text{Ca}^{2+}$  concentration within the ER lumen induces a rise in intracellular cAMP. Therefore, they proposed the SOcAMPS, in which the content of internal  $\text{Ca}^{2+}$  stores is directly connected to cAMP signaling through a process that involves STIM1. Subsequently, Nichols *et al* (113) determined that in T84 colonic cells, the  $I_{\text{sc}}$ , cAMP and PKA activity was increased under  $\text{Ca}^{2+}$ -free conditions after treatment with  $\text{Ca}^{2+}$ -releasing agonist CCh and  $\text{Ca}^{2+}$  ionophore and suppressed by pre-treatment with BAPTA-AM. Furthermore, the effects of ER  $\text{Ca}^{2+}$  store depletion on cAMP/PKA activity were attenuated by  $\text{Ca}^{2+}$  entering from the extracellular space, indicating that the production of cAMP decreased after  $\text{Ca}^{2+}$  influx. They proposed that a discrete component of the ' $\text{Ca}^{2+}$ -dependent' secretory activity in the colon is derived from cAMP generated through SOcAMPS. These studies further support the notion that  $\text{Ca}^{2+}$  and cAMP signaling may independently trigger epithelial ion transport.

**Basolateral  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels ( $\text{K}_{\text{ca}}$ ).** In GI epithelial cells,  $\text{K}^+$  channels are important in the intestinal epithelium, contribute to the stabilization of membrane voltage and provide the driving force for electrogenic anion transport (114). The concept that cholinergic agents promote the intestinal  $\text{Cl}^-$  secretion via the activation of membrane  $\text{K}^+$  conductance and maintain the cellular  $\text{Cl}^-$  transport has been widely accepted (115). Certain cholinergic agents, such as CCh, activate muscarinic receptors or acetylcholine raises the  $[\text{Ca}^{2+}]_{\text{cyt}}$ , which activates  $\text{K}_{\text{ca}}$  conductance and secondarily stimulates  $\text{Cl}^-$  secretion via the apical CFTR (116). Therefore, basolateral  $\text{K}^+$  channels hyperpolarize apical membrane potential and increase the electrical driving force for anion ( $\text{Cl}^-$  and  $\text{HCO}_3^-$ ) secretion to maintain electroneutrality.

To date, three different subtypes of  $\text{K}_{\text{ca}}$  channels expressed on colonic surface and crypt cells have been identified: Large-conductance  $\text{K}_{\text{ca}}$  channels, intermediate-conductance  $\text{K}_{\text{ca}}$  channels ( $\text{IK}_{\text{ca}}$ ) and small-conductance

$\text{K}_{\text{ca}}$  channels (117,118). Among them,  $\text{IK}_{\text{ca}}$  channels have an important role in epithelial  $\text{Cl}^-$  secretion. A selective blocker of  $\text{IK}_{\text{ca}}$  channels, clotrimazole, inhibited the  $\text{Cl}^-$  secretion in intact colonic epithelium and human colonic T84 cells (119). In addition, it has been demonstrated that activation of CFTR alone is insufficient to evoke transepithelial  $\text{Cl}^-$  secretion and that basolateral membrane  $\text{K}^+$  channels are also necessary components of the secretory response (30). Therefore, basolateral membrane  $\text{K}_{\text{ca}}$  channels represent an important potential therapeutic target to increase  $\text{Cl}^-$  secretion in patients with CF.

While the expression and function of  $\text{K}_{\text{ca}}$  channels and their role in the regulation of duodenal epithelial ion transport and DBS in the duodenal epithelium have remained elusive, it is well known that  $[\text{Ca}^{2+}]_{\text{cyt}}$  has an important role in epithelial ion transport (2,11); however, the underlying mechanisms of  $[\text{Ca}^{2+}]_{\text{cyt}}$  to induce duodenal  $\text{HCO}_3^-$  secretion, or indeed other ion transport systems, had not been explored in detail. Therefore, the functionality of  $\text{K}_{\text{ca}}$  and their role in the regulation of duodenal mucosal ion transport were explored. A previous review by the current researchers provided evidence that  $\text{IK}_{\text{ca}}$  or intermediate conductance calcium-activated potassium channel protein 4/SK4 channels are located on the basolateral side of duodenal epithelial cells and are involved in the regulation of  $\text{Ca}^{2+}$ -mediated duodenal  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretion (120). Furthermore, it was indicated that clotrimazole, a selective blocker of basolateral  $\text{IK}_{\text{ca}}$ , was able to inhibit  $\text{Ca}^{2+}$ -mediated duodenal  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretion, suggesting its potential utility as an anti-diarrheal drug for the treatment of secretory diarrhea (13,121).

**NCX.** The plasma membrane NCX is an important membrane transporter and has a critical role in the maintenance of  $\text{Ca}^{2+}$  homeostasis in a variety of tissue types (122).

NCX is a bidirectional plasma membrane transporter and in each cycle, three  $\text{Na}^+$  for one  $\text{Ca}^{2+}$  are transported in the opposite direction and this process depends on electrochemical gradients (123). The expression and function of NCX have been demonstrated in cardiomyocytes, vascular cells and neurons (124). They are able to function in a forward mode to excrete intracellular  $\text{Ca}^{2+}$  and in reverse mode to induce extracellular  $\text{Ca}^{2+}$  entry and various associated signal transduction pathways (124). NCX was previously reported to be expressed in small intestinal epithelial cells and to function in the forward mode that is involved in the absorption of  $\text{Ca}^{2+}$  into the bloodstream. However, NCX also has a role in GI epithelial anion secretion (125).

Seip *et al* (126), demonstrated an interaction between SOC and NCX in the rat colon, where the influx of  $\text{Na}^+$  across SOC serves to reduce the driving force for  $\text{Ca}^{2+}$  extrusion via the NCX and thereby maintains the increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$  during the induction of rat colonic anion secretion. Consistently, Kocks *et al* (110) reported a cross-talk between the depletion of intracellular  $\text{Ca}^{2+}$  stores and NCX, which may maintain a long-lasting increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$  to amplify  $\text{Ca}^{2+}$ -dependent colonic  $\text{Cl}^-$  secretion. While it was demonstrated that muscarinic receptor induced the activation of  $[\text{Ca}^{2+}]_{\text{cyt}}$  increases, which regulates anion secretion, the underlying mechanisms of  $\text{Ca}^{2+}$  remained largely elusive. A previous study by the current researchers determined whether NCX has a role in the regulation of duodenal mucosal anion secretion by controlling

$\text{Ca}^{2+}$  homeostasis (127). The results indicated that activation of muscarinic receptors stimulated NCX activity in a reverse mode to increase  $[\text{Ca}^{2+}]_{\text{cyt}}$  in epithelial cells, leading to  $\text{Ca}^{2+}$ -dependent  $\text{HCO}_3^-$  and  $\text{Cl}^-$  secretion (127). In conclusion, NCX has an important role in  $\text{Ca}^{2+}$ -dependent anion secretion by controlling  $\text{Ca}^{2+}$  homeostasis in GI epithelial cells.

#### 4. Associated GI diseases

**Ulcers.** Ulcers refer to mucosal injury reaching the submucosa in the GI tract (128). Peptic ulcers may develop in the stomach or proximal duodenum and at the margin of a gastroenterostomy, Meckel's diverticulum or the esophagus (128). *Helicobacter pylori* (*H. pylori*) infection, non-steroidal anti-inflammatory drugs and stress cause a large proportion of peptic ulcers (129). Since patients with ulcers usually have hyperchlorhydria, proton-pump inhibitors are used to inhibit gastric acid secretion, besides eradication of *H. pylori* infection with antibiotics (130). It is well established that GI epithelial  $\text{HCO}_3^-$  secretion is critical for defending the vulnerable epithelium against various aggressive factors (87). The mucus secreted on the surface of GI mucosa and the bicarbonate ions secreted by the GI epithelium form a mucous bicarbonate barrier (87). When  $\text{H}^+$  in gastric acid diffuses to the stomach wall, it is neutralized by  $\text{HCO}_3^-$  secreted by epithelial cells (87). In this way, the surface of the gastric mucosa remains in a neutral or partially alkaline state, preventing gastric acid and pepsin from attacking the mucosa (131). The esophagus also requires  $\text{HCO}_3^-$  secretion to protect the epithelial surface from acid reflux (132). Furthermore, normal mucus release from GI epithelium requires concurrent  $\text{HCO}_3^-$  secretion, which is essential for the release of mucin molecules and their proper expansion on the surface of epithelium as well (133). As a matter of fact, DBS, as an important protector, has been confirmed in patients with duodenal ulcer whose acid-stimulated DBS is only 41% of that of healthy subjects (94). The defect in intestinal  $\text{HCO}_3^-$  secretion has further been indicated to be a risk factor for peptic ulcer diseases (134).

Additionally, normal colonic  $\text{HCO}_3^-$  secretion is critical for the mucosal defense against bacteria in the lower GI tract (87). The luminal pH was indicated to be acidic in the colon of patients with ulcerative colitis (UC), which may be caused at least in part by disturbances in the ion transport in the inflamed colon (135). Therefore, it appears important to recover normal GI epithelial  $\text{HCO}_3^-$  secretion in patients with peptic ulcers and inflamed colon to prevent their recurrence. It is of growing interest to discover novel drugs to stimulate sufficient GI epithelial  $\text{HCO}_3^-$  secretion for mucosal protection as a potential adjuvant therapy for ulcer diseases or prevention of their recurrence.

**CF.** Epithelial  $\text{HCO}_3^-$  secretion is impaired in the GI tract of patients with CF, suggesting a pivotal role of the CFTR in mediating epithelial  $\text{HCO}_3^-$  secretion (64). Patients with CF usually have an epithelial  $\text{HCO}_3^-$  deficit. As discussed earlier, while the CFTR is mainly triggered by the cAMP/PKA pathway, most of the channels involved in GI epithelial anion secretion, including CaCC, anion exchangers,  $\text{K}_{\text{Ca}}$  and even CFTR, may be generally triggered by  $\text{Ca}^{2+}$  signaling (136). For instance, the CaCC is stimulated by  $\text{Ca}^{2+}$  ionophores and  $\text{Ca}^{2+}$ -mobilizing

secretagogues in luminal membranes of GI epithelia from subjects with or without CF (136), including ACh, CCh, histamine, bradykinin, ATP and UTP (77,78). Furthermore, a previous study by the current researchers demonstrated that adult CF mice exhibited a partial CFTR-independent duodenal  $\text{HCO}_3^-$  secretion in response to CCh, although they did not display CFTR-dependent  $\text{Cl}^-$  secretion (58). More recently, a study by the current researchers demonstrated that caffeine stimulated  $\text{Ca}^{2+}$ -dependent duodenal anion secretion, which was able to be attenuated by selective CaCC blockers, suggesting that the CaCC is one of the downstream effectors of  $\text{Ca}^{2+}$  signaling (82). Therefore, after the cAMP-activated CFTR is impaired in CF, targeting the  $\text{Ca}^{2+}$ -mediated pathway may be a potential adjuvant for CF therapy. Calcium ions have a critical role in the normal functioning of the gastrointestinal system (137). Certain calcium channel blockers were used to affect all of the organs of the gastrointestinal tract and may have therapeutic efficacy against esophageal spasm, mesenteric vascular insufficiency, irritable bowel syndrome, dyskinesia of the Sphincter of Oddi and insulinoma (137); however, this requires further intensive investigation.

**Inflammatory bowel disease (IBD).** IBD, including Crohn's disease and UC, is a group of chronic inflammatory disorders of the GI tract. Diarrhea is the most highly prevalent and debilitating symptom of IBD (138). The pathogenesis of IBD is multifactorial and involves variations in patients' genome, immune response, the intestinal microbiome and environmental factors to result in an excessive and abnormal host immune response (139). However, the change of expression and/or function of epithelial ion channels and transporters may result in electrolyte retention and water accumulation in the intestinal lumen, leading to diarrhea in IBD (139). IBD is a chronic inflammatory disorder with high complex endogenous inflammatory mediators, including IL- $\beta$ , tumor necrosis factor- $\alpha$ , interferon- $\gamma$ , IL-6, monochloramine and nitric oxide (140). They may act on intestinal epithelial ion transport and smooth muscle (141). Furthermore, the colon of patients with UC has an acidic luminal pH, which impairs the ion transport in the inflamed colon (142). Consistently, the expression of  $\text{Cl}^-/\text{HCO}_3^-$  exchanger SLC26A3 (DRA) was reported to be markedly decreased in the inflamed colon (143). The expression of DRA was also indicated to be absent exclusively in UC patients, indicating inadequate membrane trafficking events (144,145). Furthermore, in a recent genome-wide association study, a single-nucleotide polymorphism in the SLC26A3 gene was identified as a risk factor for UC development (146). A strong reduction in  $\text{Cl}^-$  absorption was identified in parallel with a low expression of DRA in UC colonic crypts (147). Therefore, decreased DRA expression may lead to a deficient  $\text{Cl}^-$  absorption in UC, which emphasizes the important role of DRA in UC-associated diarrhea (148).

**Congenital chloride diarrhea (CLD).** It is well established that DRA and PAT-1 are the two major transporters involved in apical  $\text{Cl}^-/\text{HCO}_3^-$  exchange in the GI tract (88,90). As mentioned above, loss of the expression and function of DRA may induce diarrheal disorders (143). However, mutations in the DRA gene that encode  $\text{Cl}^-/\text{HCO}_3^-$  exchange cause a rare diarrheal disorder named CLD, which is associated with a high stool concentration of  $\text{Cl}^-$ , metabolic alkalosis and physiologic



evidence of an absence of  $\text{Cl}^-/\text{HCO}_3^-$  exchange in the colon and ileum (149). Therefore, the characteristics of patients with CLD include voluminous diarrhea, massive loss of  $\text{Cl}^-$  via the stool and metabolic alkalosis. Furthermore, the pH of the ileocolonic lumen in patients with IBD has been reported to be reduced due to limited  $\text{HCO}_3^-$  secretion (146). Since DRA serves as the major luminal intestinal  $\text{Cl}^-/\text{HCO}_3^-$  exchanger responsible for bulk intestinal  $\text{Cl}^-$  absorption and  $\text{HCO}_3^-$  secretion, DRA deficiency is one of the important factors in the pathogenesis CLD and IBD (147). Therefore, based on the reported  $\text{Ca}^{2+}$ -mediated inhibition of colonic DRA, it may be speculated that inhibition of intracellular  $\text{Ca}^{2+}$  signaling may have therapeutic potential to improve excessive fluid secretion, thereby providing a novel research direction for the treatment of CLD and IBD.

## 5. Conclusion

GI epithelial anion and fluid transport have critical roles in maintaining normal physiological functions in the GI tract. Defective GI epithelial anion secretion has been critically implicated in the pathophysiology of ulcer diseases, CF, intestinal inflammation, diarrhea/constipation and even metabolic acidosis. Similarly,  $[\text{Ca}^{2+}]_{\text{cyt}}$  also has a critical role in the regulation of digestive functions. GI epithelial anion secretion is known to be controlled by several neuro-humoral factors, including  $\text{PGE}_2$ , ACh and 5-HT. These factors mediate epithelial anion secretion mainly through  $\text{Ca}^{2+}$ , cAMP and cGMP signaling pathways. Although multiple interactions exist between  $\text{Ca}^{2+}$  signaling and the cAMP pathway to trigger GI epithelial anion secretion, growing lines of evidence indicated that  $\text{Ca}^{2+}$  signaling may mediate epithelial anion secretion in a cAMP-independent manner.  $\text{Ca}^{2+}$  signaling modulates GI epithelial anion secretion through acting on CFTR, CaCC,  $\text{Cl}^-/\text{HCO}_3^-$  exchanger, SOC,  $\text{K}_{\text{ca}}$  and NCX. It was previously assumed that those channels and transporters involved in GI epithelial secretion of  $\text{Cl}^-$  and  $\text{HCO}_3^-$  are identical; however, emerging evidence suggests they are different. While cAMP may be a critical factor in CFTR-mediated  $\text{Cl}^-$  secretion,  $\text{Ca}^{2+}$  signaling may have a critical role in  $\text{Cl}^-/\text{HCO}_3^-$ -mediated  $\text{HCO}_3^-$  secretion. Elucidation of the precise regulatory mechanisms of  $\text{Ca}^{2+}$ -mediated GI epithelial  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretion will markedly enhance the current knowledge of ion and fluid transport in the GI tract. Further investigation on the differences between GI epithelial secretion of  $\text{Cl}^-$  and  $\text{HCO}_3^-$  may provide novel potential drug targets to protect the upper GI tract against ulcer diseases and promote epithelial  $\text{HCO}_3^-$  secretion.

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## Authors' contributions

WS, YH, JD, XY, JL, QD, QL and LL conceived the current review article. JX and RX were responsible for the collection and assembly of the articles/published data for inclusion and interpretation in this review. All authors were involved in the writing of the manuscript. All authors have read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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