Function of K⁺–Cl⁻ Cotransporters in the Acid Secretory Mechanism of Gastric Parietal Cells

Takuto Fuji†, Kyosuke Fujita, Noriaki Takeuchi, and Hideki Sakai

Department of Pharmaceutical Physiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama; 2630 Sugitani, Toyama 930–0194, Japan.

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Gastric proton pump (H⁺, K⁺-ATPase) secretes H⁺ of acid (HCl) via the luminal membrane of parietal cells. For the HCl secretion, Cl⁻- and K⁺-transporting proteins are required. Recent our studies have demonstrated that K⁺–Cl⁻ cotransporters (KCC3a and KCC4) are expressed in gastric parietal cells. KCC3a is associated with Na⁺, K⁺-ATPase in the basolateral membrane, and KCC4 is associated with H⁺, K⁺-ATPase in the apical canalicular membrane. This paper summarizes the functional association between KCCs and P-type ATPases and the contribution of these complexes to acid secretion in gastric parietal cells.

Key words: gastric acid secretion; K⁺–Cl⁻ cotransporter; H⁺, K⁺-ATPase; Na⁺, K⁺-ATPase; gastric parietal cell

1. TRANSPORT OF K⁺ AND CL⁻ IN GASTRIC PARIETAL CELLS

Gastric acid (HCl) is secreted by the parietal cell in stomach. Parietal cells secrete 1–2 l per day of gastric juice which contains about 160 mm HCl. Gastric acid secretion is accompanied with dramatic morphological changes of the parietal cells. In resting parietal cells, tubulovesicles are present in intracellular compartments underlying the apical canalicular membrane and form a reticulated meshwork. Upon stimulation, tubulovesicles translocate and connect with the apical canalicular membrane, resulting in massive acid secretion.¹,²

Gastric H⁺ secretion is mediated by proton pump (H⁺, K⁺-ATPase), which belongs to the family of P-type ATPases including Na⁺, K⁺-ATPase and Ca²⁺-ATPase. The H⁺, K⁺-ATPase actively transports H⁺ and K⁺ in opposite directions to generate in excess of a million-fold gradient across the membrane under physiological conditions. This proton secretion is coupled with Cl⁻ efflux across the luminal membrane. So far several Cl⁻ channels, such as cystic fibrosis transmembrane conductance regulator (CFTR),³ CLIC-6 (parachin)⁴,⁵ and SLC26A9⁶ have been reported to exist in gastric parietal cells and to be candidates for Cl⁻ secretion. CLC-2 was also proposed as a candidate for Cl⁻ secretion.⁷ However, CLC-2 knockout mice showed normal gastric acid secretion⁸ and no significant expression of CLC-2 protein was observed in the gastric mucosa of rats and humans.⁹

For maintaining H⁺, K⁺-ATPase activity in the luminal membrane of gastric parietal cells, the K⁺ recycling across the membrane is necessary. Heteromeric KCNQ1/KCNE2 channels⁴,¹¹ and the Kir4.1 channel¹²,¹³ have been postulated as candidates for this K⁺ transport.

Electroneutral K⁺–Cl⁻ cotransporters (KCCs) are secondary active symporters, which belong to a cation–chloride cotransporter gene family (SLC12). KCCs contribute to transepithelial transport and to the regulation of cell volume.¹⁴–¹⁶ At least four KCC isoforms (KCC1—KCC4) have been identified to date. KCC1 is widely expressed. KCC2 is specifically expressed in neuronal cells. KCC3 has two distinct amino terminal domains, generated by the use of two different first coding exons, 1a (for KCC3a) and 1b (for KCC3b). The longer isoform KCC3a is expressed in several epithelial type cells. In contrast, the shorter isoform KCC3b is abundantly expressed in kidney. KCC4 is mainly located in epithelial cells.

If KCCs are present in the luminal membrane of gastric parietal cells, they may be functionally coupled with H⁺, K⁺-ATPase and contribute to HCl secretion. Recently we found that KCC3a and KCC4 were expressed in the basolateral and apical canalicular membranes of gastric parietal cells, respectively¹⁸,¹⁹ (Fig. 1). In this review, we summarize the function of these KCCs in gastric parietal cells.

2. EXPRESSION AND FUNCTION OF KCC3a IN THE BASOLATERAL MEMBRANE OF GASTRIC PARIETAL CELLS

KCC3a protein was not co-localized with H⁺, K⁺-ATPase. It was co-localized with Na⁺, K⁺-ATPase α1-subunit (α1NaK) in the basolateral membrane of gastric parietal cells (Fig. 1). Interestingly, KCC3a was abundantly expressed in the parietal cell at the luminal region of gastric glands where gastric acid secretion is more active than those at the basal region of the glands (Fig. 1). Therefore KCC3a is suggested to be involved in gastric acid secretion. In rat gastric mucosa, both KCC3a and α1NaK were highly localized in lipid rafts, a cholesterol-enriched microdomain, and KCC3a was co-immunoprecipitated with α1NaK. A KCC inhibitor (DIOA) decreased the ATP-hydrolyzing activity of Na⁺, K⁺-ATPase in rat gastric mucosa in which KCC3a was predominantly expressed, while it had no effect on the activity in rat kidney in which KCC3b was predominantly expressed. Therefore, KCC3a may be coupled with α1NaK and up-regulates the pump activity in lipid rafts of the cells. In the tetracycline-inducing expression system of KCC3a in...
In the luminal region of gastric glands, KCC3a and KCC4 are expressed in basolateral and apical membranes of parietal cells, respectively. In the basal region, both KCC3a and KCC4 are absent. H\(^+\), K\(^+\)-ATPase (HK) and Na\(^+\), K\(^+\)-ATPase (NaK) are expressed both in the luminal and basal regions of glands. It is noted that the luminal parietal cells more actively secrete acid than do the basal parietal cells.

In LLC-PK1 cells which do not express KCC3a, Na\(^+\), K\(^+\)-ATPase (NaK) is mainly distributed in non-rafts. The exogenous expression of KCC3a recruits Na\(^+\), K\(^+\)-ATPase into lipid rafts and up-regulates Na\(^+\), K\(^+\)-ATPase activity. Flotillin-2 is a marker protein of lipid rafts.

KCC4 protein was expressed in the parietal cell and co-localized with H\(^+\), K\(^+\)-ATPase (Fig. 1). As the case for KCC3a, KCC4 was also abundantly expressed in the parietal cell at the luminal region of gastric glands (Fig. 1). Two types of vesicles can be prepared from hog gastric mucosa; one is intracellular tubulovesicles (TV) and another is stimulation-associated vesicles (SAV) derived from apical surface membrane. In TV, DIOA dose not inhibit the ATP-dependent Cl\(^-\) uptake in SAV (C) (Fig. 3). In TV, DIOA does not inhibit the ATP-dependent Cl\(^-\) uptake in SAV (C). In TV, DIOA dose not inhibit the ATP-dependent Cl\(^-\) uptake, suggesting that Cl\(^-\) transporting protein other than KCCs may exist in TV.

NH\(_2\)-terminus of KCC3a may be a key region for association with Na\(^+\), K\(^+\)-ATPase.\(^{20}\)

3. EXPRESSION AND FUNCTION OF KCC4 IN THE APICAL CANALICULAR MEMBRANE OF GASTRIC PARIETAL CELLS

KCC4 protein was expressed in the parietal cell and co-localized with H\(^+\), K\(^+\)-ATPase (Fig. 1). As the case for KCC3a, KCC4 was also abundantly expressed in the parietal cell at the luminal region of gastric glands (Fig. 1). Two types of vesicles can be prepared from hog gastric mucosa; one is intracellular tubulovesicles (TV) and another is stimulation-associated vesicles (SAV) derived from apical surface membrane.\(^{20}\) H\(^+\), K\(^+\)-ATPase was expressed both in SAV and TV. Rab11 was mainly expressed in TV, whereas ezrin and \(\beta\)-actin were mainly expressed in SAV. KCNQ1 and CFTR were expressed in TV but not in SAV. Interestingly KCC4 was abundantly expressed in SAV but not in TV, indicating that KCC4 is present in the apical canalicular membrane but not in tubulovesicles of gastric parietal cells. In SAV, KCC4 was co-immunoprecipitated with H\(^+\), K\(^+\)-ATPase.\(^{19}\)

In SAV, the ATP-dependent Cl\(^-\) uptake was inhibited by DIOA and SCH28080 which is an inhibitor of H\(^+\), K\(^+\)-ATPase (Fig. 3). In TV, the Cl\(^-\) uptake was not inhibited by DIOA, but inhibited by SCH28080 (Fig. 3). Therefore, KCC4-mediated secondary Cl\(^-\) transport is present only in SAV.

Interestingly, in SAV, the ATP-dependent H\(^+\) uptake and the ATP-hydrolyzing activity of H\(^+\), K\(^+\)-ATPase were significantly inhibited by DIOA, suggesting that H\(^+\), K\(^+\)-ATPase activity is positively regulated by KCC4 (Fig. 3). The functional association between KCC4 and H\(^+\), K\(^+\)-ATPase was also observed in the KCC4-H\(^+\), K\(^+\)-ATPase co-expressing HEK293 cells; KCC4 was co-immunoprecipitated with H\(^+\), K\(^+\)-ATPase, and the H\(^+\) transport activity of H\(^+\), K\(^+\)-ATPase was up-regulated by KCC4.

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**Fig. 1.** Expression Patterns of KCC3a and KCC4 in Parietal Cells of Gastric Glands

In the luminal region of gastric glands, KCC3a and KCC4 are expressed in basolateral and apical membranes of parietal cells, respectively. In the basal region, both KCC3a and KCC4 are absent. H\(^+\), K\(^+\)-ATPase (HK) and Na\(^+\), K\(^+\)-ATPase (NaK) are expressed both in the luminal and basal regions of glands. It is noted that the luminal parietal cells more actively secrete acid than do the basal parietal cells.

**Fig. 2.** Functional Association between KCC3a and Na\(^+\), K\(^+\)-ATPase in Lipid Rafts

In LLC-PK1 cells which do not express KCC3a, Na\(^+\), K\(^+\)-ATPase (NaK) is mainly distributed in non-rafts. The exogenous expression of KCC3a recruits Na\(^+\), K\(^+\)-ATPase into lipid rafts and up-regulates Na\(^+\), K\(^+\)-ATPase activity. Flotillin-2 is a marker protein of lipid rafts.

**Fig. 3.** Functional Association between KCC4 and H\(^+\), K\(^+\)-ATPase in the Apical Canalicular Membrane

KCC4 is expressed in stimulation-associated vesicles (SAV) derived from the apical canalicular membrane, while it is not significantly expressed in tubulovesicles (TV) derived from intracellular tubulovesicles. (A) In SAV, the ATP-dependent Cl\(^-\) uptake by KCC4 is inhibited by DIOA, and this inhibition leads to a decrease in the H\(^+\) uptake by H\(^+\), K\(^+\)-ATPase (HK). (B) Inhibition of H\(^+\), K\(^+\)-ATPase by SCH28080 decreases the ATP-dependent Cl\(^-\) uptake. In TV, DIOA does not inhibit the ATP-dependent Cl\(^-\) uptake, suggesting that Cl\(^-\) transporting protein other than KCCs may exist in TV.
Fig. 4. Putative Models for Gastric Acid Secretion in the Resting and Stimulated States of the Parietal Cell

In the resting state, KCC4 together with H\(^+\), K\(^+\)-ATPase (HK) in the apical membrane are involved in the basal acid secretion. Upon stimulation, Cl\(^-\)-channels (CFTR, CLIC-6, SLC26A9), K\(^+\)-channels (KCNQ1/KCNE2, Kir4.1) in tubulovesicles and KCC4 in the apical canalicular membrane are involved in the KCl transport for massive gastric acid secretion. In the basolateral membrane, KCC3a itself may negatively regulate gastric acid secretion. Na\(^+\)-ATPase negatively contributes to the acid secretion via the activation of basolateral KCC3a and positively contributes via the activation of the apical KCC4 and via the increased activity of luminal K\(^+\)-channels. To supply Cl\(^-\) from blood into the cytosol, the presence of Cl\(^-\)/HCO\(_3\)^- exchanger, anion exchanger 2, in the basolateral membrane of the parietal cell has been postulated.

4. ION CHANNELS AND TRANSPORTERS INVOLVED IN GaSTRIC ACID SECRetION

In our study, CFTR and KCNQ1/KCNE2 were expressed mainly in tubulovesicles. It has been reported that CLIC-6 is distributed throughout the cytosol\(^5\) and that SLC26A9\(^6\) is mainly in tubulovesicles. It has been reported that CLIC-6 is localized in tubulovesicles. Kir4.1 and KCNQ1 are also resided in tubulovesicles and are co-immunoprecipitated with H\(^+\), K\(^+\)-ATPase in resting and stimulated parietal cells.\(^13\) The distribution pattern of these K\(^+\) and Cl\(^-\) channels are apparently different from that of KCC4 in the apical canalicular membrane. Knockout mice of KCNQ1/KCNE2\(^21-23\) and SLC26A9,\(^6\) and ΔF508-deficient mice of CFTR\(^3\) exhibit a significant decrease in gastric acid secretion during acid stimulation. A highly selective KCNQ1 channel inhibitor (HMR 1556) did not inhibit basal acid secretion, while it significantly inhibited forskolin-stimulated acid secretion.\(^13\) Secretagogue-induced H\(^+\), K\(^+\)-ATPase activity in isolated gastric glands from mice could be significantly reduced by either glibenclamide or CFTR-inh172, an inhibitor of CFTR.\(^3\) Thus, these K\(^+\) and Cl\(^-\) channels in tubulovesicles are important for the stimulated acid secretion. On the other hand, KCC4 is located in the apical canalicular membrane in both resting and acid stimulating states but not in tubulovesicles. Additionally, the expression patterns of H\(^+\), K\(^+\)-ATPase, β-actin and ezrin did not change in both states. Therefore, we consider that the apical and tubulovesicular membranes may not mix but remain separate and distinct, when the tubulovesicular membrane is connected with the apical canalicular membrane upon stimulation (Fig. 4). Here, we propose possible roles of KCC3a and KCC4 in a putative model of acid secretion (Fig. 4): In the resting state, KCC4 together with H\(^+\), K\(^+\)-ATPase that is present in the apical membrane are involved in the basal acid secretion. Upon stimulation, CFTR, CLIC-6, SLC26A9, KCNQ1/KCNE2, Kir4.1 and KCC4 are involved in the KCl transport for massive gastric acid secretion. Recently, it has been reported that basal acid secretion was decreased in KCNQ1-deficient young mice (7- to 8-d-old).\(^22\) Therefore some KCNQ1 channels may be functionally expressed in the apical canalicular membrane and be involved in the basal acid secretion in addition to KCC4. Further, KCC3a may negatively regulate the gastric acid secretion by transporting Cl\(^-\) from cytosol to blood across the basolateral membrane of parietal cells. To supply Cl\(^-\) from blood into the cytosol, the presence of Cl\(^-\)/HCO\(_3\)^- exchanger, such as anion exchanger 2, in the basolateral membrane of the parietal cell has been postulated.\(^24\)

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