Potassium channel KCNJ15 is required for histamine-stimulated gastric acid secretion

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Yuan J, Liu W, Karvar S, Baker SS, He W, Baker RD, Ji G, Xie J, Zhu L. Potassium channel KCNJ15 is required for histamine-stimulated gastric acid secretion. Am J Physiol Cell Physiol 309: C264–C270, 2015. First published June 24, 2015; doi:10.1152/ajpcell.00012.2015.—Gastric acid secretion is mediated by the K+–dependent proton pump (H+-K+-ATPase), which requires a continuous supply of K+ at the luminal side of the apical membrane. Several K+ channels are implicated in gastric acid secretion. However, the identity of the K+ channel(s) responsible for apical K+ supply is still elusive. Our previous studies have shown the translocation of KCNJ15 from cytoplasmic vesicles to the apical membrane on stimulation, indicating its involvement in gastric acid secretion. In this study, the stimulation-associated trafficking of KCNJ15 was observed in a more native context with a live cell imaging system. KCNJ15 molecules in resting live cells were scattered in cytoplasm but exhibited apical localization after stimulation. Furthermore, knocking down KCNJ15 expression with a short hairpin RNA adenoviral construct abolished histamine-stimulated acid secretion in rabbit primary parietal cells. Moreover, KCNJ15, like H+-K+-ATPase, was detected in all of the parietal cells by immunofluorescence staining, whereas only about half of the parietal cells were positive for KCNQ1 under the same condition. Consistently, the endogenous protein levels of KCNJ15, analyzed by Western blotting, were higher than those of KCNQ1 in the gastric mucosa of human, mouse, and rabbit. These results provide evidence for a major role of KCNJ15 in apical K+ supply during stimulated acid secretion.

Gastric acid secretion is mediated by the proton pump (gastric H+-K+-ATPase) along with K+ efflux channels and Cl− channels (6, 10, 11, 26). H+,K+-ATPase is located on the cytoplasmic turobluvosomes (TVs) of parietal cells in the resting state. On stimulation by histamine or other stimuli, H+,K+-ATPase-enriched TVs fuse to the apical membrane, where H+,K+-ATPase transports H+ into the lumen in exchange for K+ at a 1:1 ratio. The activity of H+,K+-ATPase requires K+ in the lumen fluid (4, 28, 29). Continuous supply of K+ at the luminal side of the apical membrane is achieved by K+ efflux channels. Cl−, as a counter ion for H+, is secreted into the lumen through Cl− channels (21, 27). It has been well documented that K+ efflux channels are required for gastric acid secretion, but the identity of the potassium channel(s) coupled with gastric acid secretion is still elusive. Several K+ channels, including KCNQ1 (KvLQT1) (2, 7, 9, 15–17, 20, 32), KCNJ1 (ROMK/Kir1.1) (34), KCNJ2 (Kir2.1) (1, 19), KCNJ10 (Kir4.1) (5, 14, 31), and KCNJ15 (Kir4.2) (8), have been implicated in gastric acid secretion, although sharp inconsistencies, significant gaps, and questions remain to be addressed before their roles in parietal cells are understood.

KCNQ1 was the first K+ channel implicated in gastric acid production. KCNQ1 mutation in human has been known to cause long QT syndrome with serious consequences including syncope, seizure with loss of consciousness, or resuscitated cardiac arrest. Surprisingly, KCNQ1 knockout mice exhibit normal cardiac function, but their stomachs do not produce acid (17). Functional importance of KCNQ1 in acid secretion was elucidated in other studies using knockout techniques targeting KCNQ1 (17, 32) or KCNE2 (25), the β-subunit of the KCNQ1 channel complex. More evidence in support of a role of KCNJ1 in acid secretion includes localization of KCNJ1 on the apical membrane in stimulated mouse parietal cells (7) and the suppression of acid production with KCNJ1 inhibitors 293B (7) and XE-991 (34). However, the mechanism for the role of KCNJ1/KCNE2 in acid secretion is not clear, and several lines of evidence suggest that KCNJ1/E2 is not likely the K+ channel for apical K+ supply in stimulated acid secretion. First, patients with inactivating KCNJ1 mutations rarely exhibit abnormalities in gastric acid secretion (24, 35); second, KCNJ1 is expressed in only about 50% of the parietal cells (10); and third, a high concentration (1,000 IC50) of a KCNJ1 inhibitor, HMR-1556, still allowed robust stimulated acid secretion (14). KCNJ15 is implicated as an essential factor for the secretion of insulin (22), brain development (33), and acid secretion in the lung (37). KCNJ15 is also expressed in the kidney (30). In lung epithelial cells, KCNJ15 supplies K+ to the proton pump on the apical membrane. Our previous studies showed that the KCNJ15 mRNA is the most highly expressed among all K+ channels in the gastric mucosa and that this channel showed a stimulation-associated translocation onto apical membrane indicative of a role for stimulated acid secretion (8). In this article we report that KCNJ15 is expressed at a higher level than KCNJ1 in human, rabbit, and mouse gastric mucosa, that it is translocated to apical membrane on histamine stimulation, and that knocking down KCNJ15 abolishes histamine-stimulated acid secretion. These results provide evidence that KCNJ15 plays a critical role in stimulated gastric acid secretion.

MATERIALS AND METHODS

Human gastric mucosa samples. Human gastric mucosa samples were obtained from patients who underwent esophagogastrroduo-
Knockdown of KCNJ15 by short hairpin RNA. Three RNA interference (RNAi) target sequences against rabbit KCNJ15 mRNA (XM_002721258) were selected using BLOCK-iTRNAi Designer (Invitrogen). A random oligo with similar GC content but not matching any rabbit DNA sequence was used as the control. The rabbit KCNJ15 short hairpin RNA (shRNA) and control shRNA target sequences are as follows: shRNA#1, GCAATGTGAGGATCGACCTTCTACCATGT; shRNA#2, GGTACAAAGCTCAGCTGTGT; shRNA#3, GCCCATGACCTTCTACATGT; and control shRNA, GAATCGCGTACCTGACTA. The corresponding shRNA sequences were cloned into vector pDC311-mCherry (13), which is an adenoviral shuttle vector modified from pDC311 to carry an U6 promoter to drive the expression of an shRNA sequence, and an independent open reading frame (ORF) encoding mCherry to indicate the production of recombinant adenovirus. To test the efficiencies, KCNJ15 shRNA plasmids were transfected into a rabbit lung fibroblast cell line R9ab using GenJet transfection reagent. The KCNJ15 protein levels were then analyzed by Western blotting and quantitated by densitometry. The KCNJ15 signals were normalized to β-actin, and then the relative level in the control was normalized to 1. The most effective KCNJ15 shRNA plasmid was used to generate adenovirus. Adenovirus expressing KCNJ15 shRNA was used to knock down KCNJ15 in rabbit primary parietal cells.

Indirect measurement of acid secretion by isolated parietal cells. The [14C]aminopyrine (AP) uptake assay was used to measure the accumulation of aminopyrine in acidic spaces caused by the proton-pumping enzyme H+\(\cdot\)K+\(-\)ATPase. In the neutral state, AP freely equilibrates across biological membranes, but protonation of AP in an acidic environment gives it a positive charge and traps it usually. The AP uptake assay is conducted 1 or 2 days after the isolation of the parietal cells for the highly stimulated acid secretion. However, in this study, AP uptake assays were typically conducted 4 days after the isolation of parietal cells to allow the reduced expression of KCNJ15 as a consequence of RNAi delivery by the viral vector described above. Cells were either held in a resting state with the H2-receptor blocker cimetidine at a final concentration of 100 μM or stimulated with histamine 10 mM to a final concentration of 100 μM. Cultures were gently shaken for 25 min at 37°C in the presence of [14C]AP (40 nCi/ml). After the incubation, the concentration of 14C trapped in the cells and that remaining in the medium were determined by liquid scintillation counting. AP ratio is defined as the counts per minute of the cells divided by the counts per minute of the medium.

Immunofluorescence and confocal microscopy. The localization of KCNQ1 and KCNJ15 in mouse gastric mucosa was determined on mouse stomach cryosections. Mouse stomach was excised immediately after euthanasia and cut into small pieces smaller than 5 mm in any dimension for efficient fixation with 3.7% paraformaldehyde for 15 min at room temperature. After fixation, tissue was immersed in 30% sucrose and then frozen in Optimal Cutting Temperature medium (Sakura Finetek, Torrance, CA). Sections (10 μm) were prepared from these frozen blocks with a cryostat. These sections were then rehydrated in PBS, permeabilized with 0.5% Triton X-100 in PBS with 1% BSA, and stained with antibodies followed by fluorescent secondary antibodies. Primary antibodies used in this study include rabbit anti-KCNJ15 antibody (1:20; Sigma Aldrich), rabbit anti-KCNQ1 antibody (1:20; Santa Cruz), and mouse anti-β H+\(\cdot\)K+\(-\)ATPase antibody (1:50; Affinity Bioreagents). Alexa 568- and Alexa 488-conjugated secondary antibodies (1:1,000 for both) were products of Invitrogen. Images were collected with a Zeiss LSM510 confocal microscope at one airy unit pinhole with a Plan-Neofluar 40×/0.13 NA oil differential interference contrast objective. Green images were collected with an excitation of 488 nm and an emission of 500–550 nm, whereas the red images were collected with an excitation of 561 nm and an emission of 575–615 nm.
Statistical analysis. ANOVA, followed by Dunnnett’s multiple comparison tests, was performed to analyze the shRNA knockdown efficiencies. Unpaired Student’s *t*-tests were performed to analyze the differences between resting and stimulated groups in AP uptake assay. A *P* value <0.05 was considered to be significant.

RESULTS

Histamine stimulates KCNJ15 translocation. Our previous studies showed that KCNJ15 proteins were located in cytoplasmic vesicles (distinct from TVs) in resting parietal cells and in apical membrane in stimulated parietal cells, suggesting that KCNJ15, like H⁺,K⁺-ATPase, was translocated from cytoplasmic vesicles to apical membrane on stimulation. In this study, we used a live cell imaging system to provide direct evidence for the trafficking of KCNJ15 in rabbit parietal cells. KCNJ15-CFP adenovirus was used to infect rabbit parietal cells for 48 h to express KCNJ15-CFP. The distribution of KCNJ15-CFP in cells before and after histamine stimulation was monitored using an inverted Zeiss fluorescence microscope. Under normal physiology, almost the entire apical membrane of parietal cell exists as intracellular canalicular membrane. Once the parietal cells are isolated and cultured in vitro, the apical canalicular membrane becomes intracellular vacuoles that are separated from the plasma membrane. On histamine stimulation, acid would be trapped in the vacuoles and the parietal cell would swell consequently, as shown in a stimulated parietal cell infected with a control ezrin-CFP construct (Fig. 1). For cells expressing KCNJ15-CFP, images were recorded before and after histamine stimulation from the same cells (Fig. 1). Images were taken from cells exhibiting moderate swelling so that the apical vacuole membranes were distanced from the basolateral plasma membrane. A scattered cyan fluorescence was observed all over the resting parietal cells. However, in histamine-stimulated parietal cells, the cyan fluorescence was mainly found around apical membranes (intracellular vacuoles), indicating that KCNJ15 was translocated from cytoplasmic vesicles to apical membrane on histamine stimulation.

KCNJ15 knockdown diminishes histamine-stimulated acid secretion. To further determine the role of KCNJ15 in stimulated gastric acid secretion, we tested whether knocking down KCNJ15 by RNAi would have any effects on histamine-stimulated acid secretion. Three RNAi target sequences against rabbit KCNJ15 mRNA (XM_002721258) were selected with BLOCK-iTRNAi Designer software (Invitrogen). The corresponding shRNA sequences were cloned into pDC311-mCherry (13), which is an adenoviral shuttle vector modified from pDC311 to carry an U6 promoter to drive the expression of a shRNA sequence, and an independent ORF encoding mCherry to facilitate the production of recombinant adenovirus. A random oligonucleotide with similar GC content but not matching any rabbit DNA sequence was used as the control. As shown in Fig. 2A, KCNJ15 shRNA plasmids effectively knocked down the expression level of KCNJ15 in the rabbit lung fibroblast R9ab cells. Quantitative analyses showed that cells expressing KCNJ15 shRNA#1, shRNA#2, and shRNA#3 exhibited 30%, 53%, and 57% reduction in KCNJ15 protein levels, respectively, as shown in Fig. 2B. With the KCNJ15 shRNA#3 and control shRNA plasmids, recombinant adenoviruses were generated to infect primary cultures of rabbit gastric parietal cells. The KCNJ15 protein level in primary parietal cells was reduced to 50% of the control level by the recombinant adenovirus expressing KCNJ15 shRNA construct, whereas H⁺,K⁺-ATPase and KCNQ1 protein levels remained unchanged (Fig. 2, C and D). The histamine-stimulated acid secretion in parietal cells was analyzed by AP uptake assay. As shown in Fig. 2E, stimulated acid production was observed with control shRNA-expressing cells. However, cells expressing KCNJ15 shRNA did not respond to histamine stimulation.

Fig. 1. Translocation of KCNJ15-CFP adenovirus in parietal cells on histamine stimulation. Cultured rabbit primary gastric parietal cells were infected with recombinant adenovirus expressing KCNJ15-CFP or with ezrin-CFP as a control. Images were taken from the same cells before and after histamine treatment using an inverted Zeiss fluorescence microscope. CFP, cyan fluorescence protein; DIC, Differential interface contrast. Scale bar, 10 μm for all panels.
KCNJ15 is expressed in all parietal cells. Next, we examined the cellular distribution of KCNJ15 in gastric mucosa. Stomachs from whole body-perfused mice were fixed with formaldehyde. Cryosections were used to examine the expression of KCNQ1 and KCNJ15 by immunofluorescence staining. As shown in Fig. 3, KCNJ15, similar to H\textsuperscript{+},K\textsuperscript{+}-ATPase, was expressed in all parietal cells. By contrast, KCNQ1 was only detected in about half (55.3\% \pm 2.5\%, mean \pm SE, \( n = 4 \)) of the parietal cells (Fig. 3), which is consistent with a previous report(10). KCNQ1 was mainly found at the middle sections of gastric glands.

KCNJ15 is more abundantly expressed than KCNQ1 in stomach samples from human, mouse, and rabbit. To further evaluate the difference between the expression levels of KCNJ15 and KCNQ1, the endogenous protein levels of KCNJ15 and KCNQ1 in human, mouse, and rabbit stomach samples were compared by Western blotting. KCNJ15 and KCNQ1 from the plasmid transfected HEK-293 cells were used as references. Both constructs are driven by the same promoter. In an effort to achieve a similar expression level of KCNJ15 and KCNQ1 in the transfected cells, both constructs were cotransfected with a fluorescence protein to monitor and ensure similar transfection efficiencies. As shown in Fig. 4, the recombinant KCNJ15-CFP was detected at the expected molecular mass of 66 kDa (40 kDa for KCNJ15 plus 26 kDa for CFP), whereas various bands were detected for endogenous KCNJ15. In a prior study using immunoprecipitation and Western blotting (8), we demonstrated that the 80-kDa bands...
are KCNJ15-specific signals (likely dimer). With a coiled coil dimerization motif at the COOH terminus, KCNJ15 could form SDS-resistant (23) homodimers or heterodimers with other peptides such as Kir5.1 (18). With the total protein load specified in Fig. 4, KCNJ15 was detected in the gastric tissues from human, rabbit, and mouse. Assuming that the antibody is similarly reactive with all three species, the abundance of KCNJ15 in relation to the recombinant KCNJ15 in transfected HEK cells was 20.0 /H11006 7.7% for human stomach biopsy, 2.7 /H11006 0.7% for rabbit gastric mucosa, and 12.1 /H11006 3.3% for mouse gastric mucosa (n = 3 for all species). However, for the same amount of total protein load, KCNQ1 was barely detected in any of the species at extended exposures.

**DISCUSSION**

In the present study we showed that KCNJ15 was translocated to apical membrane on histamine stimulation by using a live cell imaging system. We then found that histamine-stimulated acid secretion could be abolished when KCNJ15 was knocked down by RNAi. We further showed that KCNJ15 was expressed in all parietal cells, whereas KCNQ1 was only detected in about half of the parietal cells. Consistently, the protein expression levels of KCNJ15 were higher than that of KCNQ1 in the gastric mucosa of human, rabbit, and mouse, as analyzed by Western blotting. Collectively, these results indicate the essential role of KCNJ15 in stimulated gastric acid secretion.

With fixed cell microscopy and cell fractionation studies, we previously showed that KCNJ15 translocates from cytoplasmic vesicles to apical membrane on histamine stimulation (8). In the present study we confirmed this observation under a more native condition with a live cell imaging technique. Active gastric acid secretion is mainly regulated by the membrane recycling between the apical membrane and the intracellular TVs. On removal of histamine, the densely packed microvilli collapse, and most of the proton pump-enriched membrane translocates to the intracellular TVs. Along with the proton pump, other apical membrane proteins including the K⁺ efflux channel are also expected to be removed from the apical membrane.
membrane. Similarly, on histamine stimulation, the proton pump and the K\(^+\) channel may take a similar journey from intracellular vesicles to the apical membrane. Therefore, our observation of the membrane recycling of KCNJ15 is in support of a key role of KCNJ15 in stimulated acid secretion. It is noteworthy that in resting parietal cells, KCNJ15 and the proton pump were segregated in different vesicles that can be differentiated by differential centrifugation (8). This is consistent with the observation that TVs have very low K\(^+\) conductance (12, 36). Nguyen et al. (20) reported a similar non-TV vesicle-apical membrane trafficking pattern for KCNQ1 in parietal cells. However, this pattern of KCNQ1 trafficking is brought into question by the observation from other groups that KCNQ1 is expressed in TVs (7, 9, 16).

Our RNAi study indicated that KCNJ15 plays a critical role in stimulated acid secretion. Functional studies also suggested key roles for KCNQ1/KCNE2 (17, 25, 32) and KCNJ1 (ROMK) (34) in acid secretion. As the first K\(^+\) channel suspected to play a key role in acid secretion, KCNQ1/KCNE2 was more vigorously studied than other K\(^+\) channels. Initially, one difficulty for the interpretation of the observed achlorhydria in KCNQ1 knockout mice was that these mice did not have parietal cell in the gastric epithelium (17). In an attempt to address this issue, Song et al. (32) took the 7- to 8-day-old KCNQ1 knockout mice with normal parietal cell morphology and functionality and demonstrated that KCNQ1 is required for acid secretion in the stomach of young mice. However, it has long been noticed that KCNQ1 mutation leads to deafness and cardiac diseases but is rarely associated with impaired acid secretion (35). In fact, with the use of serum gastrin level as a surrogate measurement of acid production, it was reported that only 3 of 13 KCNQ1 mutant long QT syndrome patients exhibited impaired acid production; the other 10 patients had normal serum gastrin levels, although KCNQ1 deficiency was apparent with cardiac symptoms such as syncope, seizures with loss of consciousness, or resuscitated cardiac arrest (24). In addition, a high concentration (1,000 IC\(_{50}\)) of a KCNQ1 inhibitor, HMR-1556, still allowed robust stimulated acid secretion (14). These observations led to our hypothesis that KCNJ15 may play a more important role than KCNQ1 in apical K\(^+\) supply during stimulated acid secretion.

Further evidence in support of our hypothesis comes from the comparison of the relative expression levels of the K\(^+\) channels. Our previous studies have shown that, among all K\(^+\) channels expressed in mouse gastric mucosa, KCNJ15 is the most highly expressed at the mRNA level. In this study, we found that the protein level of KCNJ15 is higher than that of KCNQ1 in human, mouse, and rabbit gastric mucosa. Importantly, whereas KCNJ15 was observed in all parietal cells, KCNQ1 was only detected in about 50% of the parietal cells. Absence of KCNQ1 in many parietal cells was also reported by Heitzmann and Warth (10). Stimulated acid secretion is a dynamic physiological process. Potassium efflux channels are tightly coupled with the proton pump. Such channels, like the proton pump, should be present in all parietal cells because the canalicular apical membrane of gastric parietal cells precludes the apical supply of K\(^+\) from adjacent cells (3, 10, 20, 36). Given that KCNQ1 is absent from many parietal cells, the seemingly critical role for KCNQ1 in acid secretion may involve mechanisms other than apical K\(^+\) supply.

One potential caveat in our study is that the KCNJ15 antibody we used in this study recognizes multiple bands in Western blotting. As a consequence, unspecific signals may be recorded in immunofluorescence staining using this antibody. However, characterization of this antibody in our previous study (8) indicated that this possibility is low for the following reasons: 1) In KCNJ15-CFP-transfected cells, the major KCNJ15-CFP band was reactive with both the KCNJ15 antibody and a CFP antibody. 2) From gastric lysate, only one major band (mutant KCNJ15 dimer) was immunoprecipitated with the KCNJ15 antibody. Therefore, this antibody may mainly react with mature dimerized KCNJ15 in native conditions (such as in procedures of immunoprecipitation and immunofluorescence staining). 3) Multiple protein bands recognized by KCNJ15 antibody in Western blotting are explained by the various processing products of KCNJ15, including the naive translational product with a signal peptide, the KCNJ15 monomer with signal peptide removed, homodimers, and heterodimers. Dimersization mediated by the coiled-coil motif found at the COOH terminus of KCNJ15 is resistant to SDS and boiling (23).

Taken together, these results support a key role for KCNJ15 in stimulated gastric acid secretion. Comparison of the expression levels and the cellular distributions of KCNJ15 and KCNQ1 suggests that KCNJ15 is more important than KCNQ1 in apical K\(^+\) supply at stimulated acid secretion. The exact roles and mechanisms for K\(^+\) channels including KCNJ15, KCNQ1, and ROMK in acid secretion and the physiology of parietal cells remain largely unknown.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

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KCNJ15 IN GASTRIC ACID SECRETION


