Acid secretion-associated translocation of KCNJ15 in gastric parietal cells

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He W, Liu W, Chew CS, Baker SS, Baker RD, Forte JG, Zhu L. Acid secretion-associated translocation of KCNJ15 in gastric parietal cells. Am J Physiol Gastrointest Liver Physiol 301: G591–G600, 2011. First published June 30, 2011; doi:10.1152/ajpgi.00460.2010.—Potassium ions are required for gastric acid secretion. Several potassium channels have been implicated in providing K+ at the apical membrane of parietal cells. In examining the mRNA expression levels between gastric mucosa and liver tissue, KCNJ15 stood out as the most highly parietal cells. In examining the mRNA expression levels between gastric mucosa and liver tissue, KCNJ15 stood out as the most highly expressed in parietal cells, as indicated by the fact that the KCNJ15 signal was shifted to a large membrane fraction that sedimented at 4,000 g. Our results demonstrate that, in nonsecreting parietal cells, KCNJ15 is stored in vesicles distinct from the H+-K+-ATPase-enriched tubulovesicles. Furthermore, upon stimulation, KCNJ15 and H+-K+-ATPase both translocate to the apical membrane for active acid secretion. Thus KCNJ15 can be added to the family of apical K+ channels in gastric parietal cells.

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POSSUM IONS ARE NEEDED in the generation of acidity by the stomach (9). For every proton pumped into the gastric lumen by the H+-K+-ATPase (H-K-ATPase), K+ is equivalently pumped into the cytosol of gastric parietal cells. To provide a continuous supply of K+ for the proper function of the proton pump it is necessary to have some leak or channel mechanism for the flow of K+ from cytoplasm to the luminal side of the proton pump. These two apical membrane transporters, the K+ channel and the H+/K+ exchange pump, together with an apical Cl− channel, account for the continuous recycling of K+ across the apical membrane and the net secretory flux of HCl acid (8, 29).

Many potassium channels or transporters have been implicated in gastric acid secretion. KCNQ1 was the first K+ channel suggested for apical K+ recycling in human and rat parietal cells (13). Soon, KCNJ10 (Kir4.1) was suggested for a similar role in rat parietal cells (12). Fujita et al. (12) also detected the mRNAs of KCNJ15 (Kir4.2) and Kir7.1 in parietal cells but failed to detect the proteins of these two channels. In addition, Cuppoletti and colleagues (5, 19) found that the mRNA of Kir2.1 (KCNJ2) is more abundant than that of Kir4.1 and Kir7.1 in rabbit gastric mucosa. They demonstrated that Kir2.1 is colococalized with H-K-ATPase and ClC-2 in parietal cells and this potassium channel can be activated by PKA and low pH. It is also noteworthy that gastric glands isolated from ROMK knockout mice (ROMK is also an inwardly rectifying K+ channel) exhibit no H-K-ATPase activity (26).

The number of K+ transporters expressed in parietal cells continued to grow, when the K+-Cl− cotransporter (KCC) 4 (KCC4, SLC12A7) was reported to be an apical protein, in both resting and stimulated cells (11). Interestingly, a KCC inhibitor suppressed H-K-ATPase activity in an in vitro assay. It is known that certain tissues [such as rat pituitary (31) and mouse cardiac cells (20)] express a large diversity of potassium channels (19). To test the hypothesis that multiple potassium channels may be involved in the apical recycling of potassium ions, we examined microarray data sets generated from mouse stomach and liver tissue. Surprisingly, microarray analysis indicated that KCNJ15 is the most highly specific K channel in the stomach. This K+ channel translocated from cytoplasm to apical membrane upon stimulation, supporting a role in the production of gastric acid.

MATERIALS AND METHODS

Animals. The procedures and treatments for handling animals were reviewed and approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo. C57BL/6 mice were anesthetized with pentobarbital before whole body perfusions were performed, with the catheter inserted into the apex of the left ventricle and the right atrium snipped to vent the perfusate (PBS with 1 mM CaCl2 and 1 mM of MgCl2, bubbled with pure oxygen). About 20 ml of perfusate was used for each mouse to achieve a complete whole-body perfusion. Mouse tissues were then stored in RNAlater (Qiagen) for the preparation of RNA or snap frozen for protein analyses.

Gastric glands (3) and gastric parietal cells (4) were prepared from New Zealand White rabbits following established methods.

Microarray data analysis. The microarray data sets for mouse stomach are taken from a recently published LASP1 knockout study (33). The dataset used here were generated from the gastric mucosa of wild-type C57BL/6 mice. The Gene Expression Omnibus (GEO, website: http://www.ncbi.nlm.nih.gov/geo/index) accession numbers for these stomach samples are GSM370907, GSM370908, and GSM370909. Similar datasets generated on the same microarray platform for livers of C57BL/6 mice were downloaded from GEO for comparison. The GEO accession numbers for these liver samples are...
GSTM388169, GSM388170, and GSM388171. The gene expression levels in all these datasets are median normalized.

qRT-PCR. Primer pairs were manually designed for kcnj15, kcnq1, polr2a, and β-actin, with the latter two housekeeping genes serving as internal references. For all primer pairs, the forward and reverse primers were designed to hybridize to sequences in different exons and to produce an amplicon of ~100 bp. Single correct PCR products were confirmed by melting-curve analysis, 3% agarose gel electrophoresis, and direct sequencing of the PCR products. All PCR reactions had efficiencies around 1.9, as determined experimentally with four-times serial-diluted samples. The primer pairs used for quantitative real-time PCR (qRT-PCR) analysis are 1) kcnj15, 5'-agcagagcgcccattgtgacagctgtgagaa-3' (sense), 5'-gctgctgctcaacccgcttgatgct-3' (antisense); 2) kcnj1, 5'-ggcgagttcaacaatccttgag-3' (sense), 5'-gattacgtctgacagggcaac-3' (antisense); 3) actb, 5'-gattgctgccacaccccttaca-3' (sense), 5'-gctacagcggacagtccgactgat-3' (antisense); and 4) polr2a, 5'-ggctgaggtctgcttgaggttgg-3' (sense), 5'-gagaaagttgctggtatggct-3' (antisense).

Tissue slices of ~5 mm thick were stored in RNA later at the time of RNA extraction with RNeasy (Qiagen, Valencia, CA). Complementary DNA was synthesized from 0.8 μg of RNA in a volume of 20 μL by use of the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). A three-step quantitative real-time PCR was performed (95°C, 30 s; 55°C, 30 s; 72°C, 10 s; 50 cycles) on an iCycler iQ real-time detection system (Bio-Rad Laboratories), using the following primer pair carrying the fragment: forward primer, 5'-GCAGACATTGCTCTGCTGTAGCAGGAGGC-3' (antisense); 2) reverse primer, 5'-gatctggcaccacacggagggagact-3' (sense), 5'-gcatacagcagacagcactgatgct-3' (antisense); and 4) polr2a, 5'-gcatacagcagacagcactgatgct-3' (antisense). Mouse total RNA was isolated from mouse gastric mucosa using the TRIzol reagent (Invitrogen). RT-PCR was performed using iScript cDNA synthesis kit (Bio-Rad Laboratories). Primers were manually designed for kcnj15, kcnq1, polr2a, and β-actin, with the latter two housekeeping genes serving as internal references. For all primer pairs, the forward and reverse primers were designed to hybridize to sequences in different exons and to produce an amplicon of ~100 bp. Single correct PCR products were confirmed by melting-curve analysis, 3% agarose gel electrophoresis, and direct sequencing of the PCR products. All PCR reactions had efficiencies around 1.9, as determined experimentally with four-times serial-diluted samples. The primer pairs used for quantitative real-time PCR (qRT-PCR) analysis are 1) kcnj15, 5'-agcagagcgcccattgtgacagctgtgagaa-3' (sense), 5'-gctgctgctcaacccgcttgatgct-3' (antisense); 2) kcnj1, 5'-ggcgagttcaacaatccttgag-3' (sense), 5'-gattacgtctgacagggcaac-3' (antisense); 3) actb, 5'-gattgctgccacaccccttaca-3' (sense), 5'-gctacagcggacagtccgactgat-3' (antisense); and 4) polr2a, 5'-ggctgaggtctgcttgaggttgg-3' (sense), 5'-gagaaagttgctggtatggct-3' (antisense).

Plasmids. Mouse total RNA was isolated from mouse gastric mucosa by use of a RNeasy mini kit (Qiagen). With this total RNA as template, KCNJ15 cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad Laboratories). The open reading frames of these plasmids were verified by sequencing. Plasmids. Mouse total RNA was isolated from mouse gastric mucosa by use of a RNeasy mini kit (Qiagen). With this total RNA as template, KCNJ15 cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad Laboratories). The open reading frames of these plasmids were verified by sequencing. Primers were manually designed for kcnj15, kcnq1, polr2a, and β-actin, with the latter two housekeeping genes serving as internal references. For all primer pairs, the forward and reverse primers were designed to hybridize to sequences in different exons and to produce an amplicon of ~100 bp. Single correct PCR products were confirmed by melting-curve analysis, 3% agarose gel electrophoresis, and direct sequencing of the PCR products. All PCR reactions had efficiencies around 1.9, as determined experimentally with four-times serial-diluted samples. The primer pairs used for quantitative real-time PCR (qRT-PCR) analysis are 1) kcnj15, 5'-agcagagcgcccattgtgacagctgtgagaa-3' (sense), 5'-gctgctgctcaacccgcttgatgct-3' (antisense); 2) kcnj1, 5'-ggcgagttcaacaatccttgag-3' (sense), 5'-gattacgtctgacagggcaac-3' (antisense); 3) actb, 5'-gattgctgccacaccccttaca-3' (sense), 5'-gctacagcggacagtccgactgat-3' (antisense); and 4) polr2a, 5'-ggctgaggtctgcttgaggttgg-3' (sense), 5'-gagaaagttgctggtatggct-3' (antisense).

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Indirect measurement of acid secretion by isolated gastric glands with 14C aminopyrine. The aminopyrine (AP) assay measures the accumulation of aminopyrine in acidic spaces caused by the proton-pumping enzyme H-K-ATPase. A detailed description of this assay was published previously (2). When cimetidine, histamine, and IBMX were used, final concentrations of these drugs were 100, 100, and 50 μM, respectively.
KCNJ15 IN GASTRIC PARIETAL CELLS

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RESULTS

KCNJ15 mRNA is highly expressed in the stomach. To identify stomach-specific potassium channels, we compared the microarray datasets generated from mouse gastric mucosa to that generated from mouse livers. The high quality of these datasets was exemplified by the facts that 1) mRNA levels of the majority of the 45,000 genes are similar between gastric mucosa and liver as represented by the housekeeping genes listed in Table 1; 2) high expression levels of stomach-specific genes were detected in the gastric mucosa compared with the liver tissues (Table 1, stomach genes); and 3) high expression levels of liver specific genes were detected in the liver tissues compared with the gastric mucosa (Table 1, liver genes).

We then examined the gene expression levels of all potassium channels and transporters with these microarray datasets. Out of the ~150 genes related to potassium transportation, the relative expression of KCNJ15 in the stomachs vs. liver stood out among all other potassium transporters and channels. The KCNJ15 mRNA level in the stomach is 33.6-fold of that in the liver (Table 1), followed by kcnk1, the stomach mRNA level of which is 20.7-fold of that in the liver. The relative expression level of KCNJ15 was much greater than all other potassium channels implicated in the apical recycling of potassium in parietal cells (Table 1): kcn2 (MIRP1), kcnj16 (kir5.1), slc12a7 (KCC4), kcnj1 (kir1.1, ROMK), kcnj1 (LTQ1), kcnj10 (kir4.1), and kcnj2 (kir4.1). High-level gene expression of KCNJ15 was also observed when the stomach microarray datasets were compared with the lymphocyte datasets generated on the same platform (data not shown). Therefore, efforts were then focused on the possible role of KCNJ15 in the parietal cells. We first sought to confirm the KCNJ15 expression level in the stomach by qRT-PCR.

Well-characterized primer pairs for kcnj16 and kcnq1 were used for qRT-PCR analysis, with housekeeping gene polrA2 as internal reference. The qRT-PCR results (Fig. 1A) indicated that KCNJ15 was most highly expressed in the kidney, followed by the stomach, and then the lung. All other tissues (including the liver) examined in this study exhibited little expression of kcnj15 mRNA, consistent with the microarray datasets. The mRNA level for kcnq1 in the stomach is apparently much lower than that of kcnj15. However, different from the microarray datasets, the qRT-PCR results suggested that the kcnq1 level in the stomach is much higher than that in the liver. The reason for this discrepancy is a subject of speculation. For example, the particular kcnq1 probe on the microarray chip might be unspecific. Higher expression of kcnj15 mRNA compared with kcnq1 mRNA was also observed when β-actin mRNA level was used as internal references (data not shown).

Expression of KCNJ15 protein in the stomach. The above results suggested the possibility that KCNJ15 is expressed in the stomach. Western blot analyses were then performed to examine the KCNJ15 protein expression in the stomach and several other tissues (Fig. 1B). Protein bands of expected ~80 kDa (32) were observed in the kidney and the stomach. Little expression was observed in the heart, the liver, the skeletal muscle, and the spleen. Unexpectedly, the brain showed high levels of KCNJ15 signal despite that the kcnj15 mRNA level was low, suggesting a slow turnover of this protein in the brain. It is of interest that the major KCNJ15 band in the lung is slightly smaller than that in the stomach and kidney.

Although KCNJ15 appeared at the expected ~80 kDa (32), the theoretical molecular mass for this protein is ~40 kDa. To be sure of the specificity of the KCNJ15 antibody, Western blots were performed with two recombinant KCNJ15 constructs: one with a CFP tag at the COOH-terminus of KCNJ15, and the other with a FLAG tag ahead of the NH2-terminal signal peptide of KCNJ15. HEK-293 cells expressing KCNJ15-CFP exhibited a KCNJ15 antibody-specific band at ~66 kDa (Fig. 2A), as expected from the peptide sizes of KCNJ15 (40 kDa) and CFP (26 kDa). Importantly, this 66-kDa band was the major signal detected by the green fluorescent protein (GFP)
antibody (Fig. 2A), which recognizes GFP and its derivatives including CFP. The FLAG-tagged KCNJ15 construct exhibited signals at two slightly different sizes, which were specifically detected by KCNJ15 antibody, compared with the nontransfected cells (Fig. 2B). The larger product was detected by a KCNJ15 antibody, immunoprecipitation was performed with FLAG antibody (Fig. 2Bfected cells (Fig. 2B)). The larger product was detected by a KCNJ15 antibody, compared with the nontransfected cells (Fig. 2B).

**Table 1. Microarray analysis of gene expression in mouse stomachs and livers**

<table>
<thead>
<tr>
<th>GenBank Accession No.</th>
<th>Gene Description</th>
<th>Mouse Stomach (n = 3)</th>
<th>Mouse Liver (n = 3)</th>
<th>Stomach/Liver P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_001039057.1</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 15 (Kcnj15)</td>
<td>32.62 ± 4.31</td>
<td>0.97 ± 0.04</td>
<td>33.57 ± 0.002</td>
</tr>
<tr>
<td>NM_008430.1</td>
<td>Potassium voltage-gated channel, subfamily K, member 1 (Kcnk1)</td>
<td>21.81 ± 3.40</td>
<td>1.05 ± 0.01</td>
<td>20.69 ± 0.004</td>
</tr>
<tr>
<td>NM_134110.1</td>
<td>Potassium Isk-related subfamily, gene 2 (Kcne2)</td>
<td>17.86 ± 4.07</td>
<td>1.06 ± 0.04</td>
<td>16.81 ± 0.015</td>
</tr>
<tr>
<td>NM_010604.3</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 16 (Kcnj16)</td>
<td>10.87 ± 0.47</td>
<td>1.20 ± 0.03</td>
<td>9.09 ± 0.000</td>
</tr>
<tr>
<td>NM_011390.2</td>
<td>Solute carrier family 12, member 7 (Slc12a7, Kcc4)</td>
<td>8.15 ± 1.16</td>
<td>3.30 ± 0.23</td>
<td>2.47 ± 0.015</td>
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<tr>
<td>NM_019659.2</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 1 (Kcnj1)</td>
<td>0.82 ± 0.03</td>
<td>0.80 ± 0.02</td>
<td>1.03 ± 0.545</td>
</tr>
<tr>
<td>NM_008434.2</td>
<td>Potassium voltage-gated channel, subfamily Q, member 1 (Kcnq1)</td>
<td>0.98 ± 0.07</td>
<td>0.97 ± 0.02</td>
<td>1.00 ± 0.957</td>
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<tr>
<td>NM_020269.3</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 10 (Kcnj10)</td>
<td>0.99 ± 0.01</td>
<td>1.05 ± 0.05</td>
<td>0.94 ± 0.259</td>
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<tr>
<td>NM_008425.2</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 2 (Kcnj2)</td>
<td>1.06 ± 0.10</td>
<td>1.20 ± 0.05</td>
<td>0.89 ± 0.299</td>
</tr>
<tr>
<td>NM_019468.1</td>
<td>Glucose-6-phosphate dehydrogenase 2 (G6pd)</td>
<td>0.85 ± 0.03</td>
<td>0.88 ± 0.06</td>
<td>0.96 ± 0.669</td>
</tr>
<tr>
<td>NM_138953.2</td>
<td>Elongation factor RNA polymerase II 2 (Eif2)</td>
<td>2.53 ± 0.09</td>
<td>2.25 ± 0.20</td>
<td>1.13 ± 0.263</td>
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<tr>
<td>NM_177342.3</td>
<td>TAF5 RNA polymerase II, TATA box binding protein (TBP)- associated factor (Taf5)</td>
<td>1.23 ± 0.07</td>
<td>1.14 ± 0.05</td>
<td>1.08 ± 0.377</td>
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<tr>
<td>NM_019647.5</td>
<td>Ribosomal protein L21 (Rpl21)</td>
<td>1.52 ± 0.26</td>
<td>1.27 ± 0.11</td>
<td>1.20 ± 0.428</td>
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<tr>
<td>NM_011297.2</td>
<td>Ribosomal protein S24 (Rps24)</td>
<td>319.49 ± 7.38</td>
<td>274.73 ± 3.48</td>
<td>1.16 ± 0.005</td>
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<tr>
<td>NM_007490.2</td>
<td>alcohol dehydrogenase 1 (class I) (Adh1)</td>
<td>13.06 ± 2.91</td>
<td>439.14 ± 2.42</td>
<td>0.03 ± 0.000</td>
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<tr>
<td>NM_011996.2</td>
<td>Alcohol dehydrogenase 4 (class II), pi polypeptide (Adh4)</td>
<td>0.79 ± 0.04</td>
<td>85.77 ± 2.70</td>
<td>0.01 ± 0.000</td>
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<tr>
<td>NM_021282.2</td>
<td>Cytochrome P450, family 2, subfamily e, polypeptide 1 (Cyp2e1)</td>
<td>0.97 ± 0.04</td>
<td>748.98 ± 41.13</td>
<td>0.00 ± 0.000</td>
</tr>
<tr>
<td>NM_009724.2</td>
<td>ATPase, H+K+ exchanging, beta polypeptide (Atp4b)</td>
<td>488.02 ± 23.11</td>
<td>0.87 ± 0.05</td>
<td>558.64 ± 0.000</td>
</tr>
<tr>
<td>NM_018731.2</td>
<td>ATPase, H+K+ exchanging, gastrin, alpha polypeptide (Atp4a)</td>
<td>485.91 ± 23.81</td>
<td>1.15 ± 0.01</td>
<td>422.64 ± 0.000</td>
</tr>
<tr>
<td>NM_025973.3</td>
<td>Progastricins (pepsinogen C) (Pgc)</td>
<td>547.01 ± 34.29</td>
<td>1.02 ± 0.07</td>
<td>538.65 ± 0.000</td>
</tr>
</tbody>
</table>

Gene expression levels (sample mean ± SE) shown were median normalized. The Gene Expression Omnibus (GEO, website: http://www.ncbi.nlm.nih.gov/geo/index) accession numbers are GSM370907, GSM370908, GSM370909 for stomach samples and GSM388169, GSM388170, GSM388171 for liver samples. Stomach/Liver values are fold difference of gene expression levels (sample mean) between mouse stomachs and mouse livers. P values were determined by 2-tailed Student t-test.

could be glycosylation: two theoretical N-glycosylation sites in KCNJ15 sequence have been cited (21, 22), yet no experimental evidence was given. To provide experimental evidence for N-glycosylation, gastric gland lysates were treated with PNGase F and analyzed by Western blot. According to data presented in Fig. 3, H-K-ATPase β showed a clear shift from the broad 60- to 80-kDa band to ~32 kDa after PNGase F treatment, whereas no change was detected for KCNJ15, indicating that this channel protein is not significantly N-glycosylated.

**Cellular and subcellular distribution of KCNJ15 in the gastric glands.** Gastric glands are composed mainly of three types of cells: parietal cells, chief cells, and mucous neck cells. Immunofluorescence staining was performed with resting glands to identify the cell type(s) expressing KCNJ15 and location within the cells. When glands were stained with KCNJ15 antibody and an antibody exclusive for parietal cells (H-K-ATPase β subunit), it was clear that positive signals for both antibodies existed in the same cells, indicating the expression of KCNJ15 in parietal cells (Fig. 4A). KCNJ15 staining appeared as variable sized puncta throughout the parietal cell.
It was also noticed that some H-K-ATPase-positive cells exhibited lower KCNJ15 signals. The punctate KCNJ15 staining was distinct from the H-K-ATPase staining, recalling the early observation that there is little potassium channel activity on the H-K-ATPase-rich tubulovesicles (29).

Glands were also costained with an antibody for pepsinogen C as well as the KCNJ15 antibody (Fig. 4B), to test whether KCNJ15 is expressed in the pepsinogen-secreting chief cells. Results indicated that some chief cells expressed KCNJ15 whereas others did not. It appeared that ∼50% chief cells were KCNJ15 positive whereas ∼80% parietal cells were expressing KCNJ15. No KCNJ15 expression was detected in the mucous neck cells (Fig. 4C) identified by positive GSII staining (7, 35).

To further characterize the subcellular localization of the channel protein, dual staining of KCNJ15 and Na\(^{+}\)-K\(^{+}\)-ATPase was performed. With Na\(^{+}\)-K\(^{+}\)-ATPase as a marker for basolateral membrane of the gastric epithelial cells (25), it is clear that most of the KCNJ15 signal is intracellular or associated with the "intracellular" canaliculal apical membrane structures (Fig. 4D). Only a very small amount of KCNJ15 signal was found close to Na-K-ATPase at the basolateral membrane. The lack of KCNJ15 association with basolateral membrane is further supported by the histamine/SCH28080-treated parietal cells (Fig. 6C), where the basolateral membrane is clearly separated from apical membrane and KCNJ15 signal was not detected on basolateral membrane.

For further insight about subcellular localization of KCNJ15, dual staining of KCNJ15 and F-actin was performed (Fig. 4E). In gastric glands, F-actin signal is dense at the canaliculal apical membrane of parietal cells, in addition to relatively weaker signal at other locations of plasma membrane. KCNJ15 was found in these parietal cells, but exhibiting a distinct pattern compared with F-actin staining.

To examine the KCNJ15 in intact tissue, cryosections of mouse gastric mucosa were dual stained for KCNJ15 and H-K-ATPase (Fig. 5). KCNJ15 was detected in all H-K-ATPase-positive parietal cells. Similar to the observations made with rabbit glands, KCNJ15 was also found in other cells. In mouse gastric mucosa, the KCNJ15 seemed to be better colocalized with H-K-ATPase compared with rabbit glands. This may be explained by the differences in sample preparation or differences in species.
Stimulation-associated translocation of KCNJ15 in the parietal cells. The fact that membrane protein KCNJ15 existed in parietal cells, and most of the KCNJ15 not on the basolateral membrane, suggested some role for this channel, other than basolateral K^+ regulation, in the physiology of parietal cells. Experiments were then performed with cultured parietal cells to ascertain whether KCNJ15 channels undergo stimulation-dependent translocation, similar to the recycling pathway well known for the H-K-ATPases (10). Upon culture, the parietal cells take on a modified morphology convenient for the studies of membrane trafficking: the invaginated apical canalicular membrane becomes completely sequestered to form intracellular vacuoles so that the apical membrane and basolateral membranes remain distinctly separated (4).

In some cases parietal cells were treated with cimetidine to maintain the resting state. In typical resting cells, H-K-ATPase exhibited staining throughout the cytosol because it is mainly present on the small cytoplasmic tubulovesicles. In these cells, KCNJ15 staining also appeared cytosolic although it was distinct from the H-K-ATPase staining (Fig. 6A). In cells treated with histamine and IBMX to achieve a maximum stimulation of acid secretion, HCl and water are pumped into the apical vacuoles. Without a mechanism to vent the acid, the vacuoles continue expanding until they occupy the majority of space in the cell. Under this condition, both H-K-ATPase and KCNJ15 appeared on the apical membrane (Fig. 6B). To exclude the possibility that the KCNJ15 staining pattern in stimulated parietal cells was a result of KCNJ15 being close to the apical membrane instead of being incorporated into it, cells were stimulated with histamine/IBMX in the presence of a proton pump inhibitor SCH28080. This treatment allows the trafficking and assembly of the machineries required for acid secretion without the actual production of acid since the pump is effectively inhibited by SCH28080 (1). Under this condition,
as demonstrated in Fig. 6C, there was a clear translocation of H-K-ATPase from cytoplasmic tubulovesicles onto the apical vacuolar membrane. Since acid secretion was inhibited by SCH28080, the expansion of the apical vacuoles was rather limited, which allowed a clear observation of the translocation and apparent colocalization of both H-K-ATPase and KCNJ15.

Fractionation of the different membranes by differential centrifugation was performed as an alternative test for the stimulation-associated translocation of KCNJ15. Isolated gastric glands were either treated with cimetidine or histamine/IBMX to achieve the two distinct physiological states: resting or stimulated. The secretory status of the glands was measured by the [14C]AP-accumulation assay (Fig. 7A). The glands were then homogenized and membranes of different sizes were fractionated by differential centrifugation. Plasma membranes including the canalicular apical membrane sediment at a lower centrifugal force whereas the smaller tubulovesicle membranes sediment at higher speed. Similar portions of each pellet and the final supernatant from both resting and stimulated preparations were analyzed by Western blots probed for signals of H-K-ATPase, KCNJ15, and β-actin (Fig. 7B). A clear redistribution of H-K-ATPase accompanied stimulation of the gastric glands. In the resting preparations, the majority of the H-K-ATPase signal was found in the smaller and/or less dense P3 microsomal membrane fraction, whereas the stimulated preparations had a large increase in H-K-ATPase in the larger and/or more dense P1 membrane fraction.

The KCNJ15 blot was a surprise. In both resting and stimulated preparations the KCNJ15 signal was detected in the larger membrane P1 and in the supernatant S3, but not in P2 or P3. The KCNJ15 signal was strong in resting S3, but weak in the stimulated S3, with a concomitant increase of KCNJ15 signal in the stimulated P1 fraction. Given the fact that KCNJ15 has a transmembrane domain in its peptide sequence, we considered the possibility that this protein may exist in a light vesicular membrane in the S3 fraction. Therefore, the S3 fractions from both resting and stimulated preparations were centrifuged for an extended 4 h at 100,000 g. The resulting pellets, designated P4, and supernatants (S4) were analyzed by Western blot for KCNJ15. Most of the KCNJ15 signal was found in the P4 pellet, confirming that the KCNJ15 vesicles are distinct from the H-K-ATPase enriched tubulovesicles. These fractionation results and the immunofluorescence staining results suggested a stimulation associated translocation of KCNJ15 from a light vesicular membrane onto the apical membrane of parietal cells.

DISCUSSION

Discovery of KCNJ15 in the gastric parietal cells. Sachs’s group (17) pioneered the microarray study on gastric parietal cells. In comparing the gene expressions between purified parietal cells and gastric mucosa, the KCNQ1 mRNA level in pure parietal cells was found to be 7.66-fold higher than in total gastric mucosa, whereas other K+ channels showed little or no increase in comparative mRNA. One drawback of this approach is that any protein highly expressed in cells other than parietal cells might be missed even if they are also highly expressed in parietal cells. This is exactly the case for KCNJ15,
which is expressed in both parietal cells and chief cells (Fig. 4B) and may be expressed in surface mucous cells (see Ref. 12). Moreover, from the data of Lambrecht et al. (17), the overall KCNJ15 signal seemed to be higher in parietal cells than that in other gastric cells, with KCNJ15 mRNA in pure parietal cells being 1.84-fold over that in total gastric mucosa.

We took a different approach when analyzing the microarray data that cover the whole mouse genome. The gene expression levels of the 45,000 transcripts were compared between gastric mucosa and a different tissue. KCNJ15 clearly stood out among other Kᵢ/Hᵢ channels in this comparison with a 33-fold higher expression level in the gastric mucosa. Confirmed by qRT-PCR analysis, this observation suggested specific expression of KCNJ15 in gastric mucosa. Immunofluorescence staining of stomach cryosections, isolated gastric glands, and parietal cells suggested that KCNJ15 was expressed in both parietal cells and chief cells, but not mucous neck cells.

It is noteworthy that KCNJ15 has been implicated in acid secretion in pulmonary epithelial cells, which use a nongastric H-K-ATPase to generate a low pH and KCNJ15 to recycle K⁺ at the apical membrane (16, 30). In these acid-secreting cells, the KCNJ15 channels are resistant to low pH, with mild inhibition seen after a long-time treatment.

A new member in the family of parietal cell apical K⁺ channels. In this study, evidence is presented to support a role for KCNJ15 in stimulated gastric acid secretion. In resting parietal cells, KCNJ15 is mainly located in the cytoplasm as indicated by immunofluorescence staining. But this cytoplasmic staining is clearly different from that of H-K-ATPase, according to both immunofluorescence staining results and the cellular fractionation results: whereas H-K-ATPase is mainly found in the P3 fraction, KCNJ15 was not detected in P3. Instead, most of the KCNJ15 remained in the “classical” S3 supernatant. Only after an extended centrifugation did the KCNJ15 sediment in newly designated P4 fraction. The separation of two types of small vesicles with distinct sedimentation properties may allow interesting studies to elucidate other differences between these two types of vesicles. The KCNJ15 signal found in the resting P1 fraction may come from other gastric cells, or even entrapment of KCNJ15 in larger membrane fragments. Nevertheless, upon stimulation, there is a clear translocation of KCNJ15 from a cytosolic compartment to the fraction rich in apical membranes. This stimulation-associated translocation strongly suggests a role for KCNJ15 although more direct evidence is needed.

A brief review of Kᵢ/Hᵢ channels in parietal cells. Several other Kᵢ/Hᵢ channels have been implicated in the apical K⁺ recycling. Although the initial study on KCNQ1 (13) suggested its colocalization with H-K-ATPase (namely, every parietal cell expressed KCNQ1 on apical membrane and tubulovesicles), close examination of immunostained tissue sections suggests that many parietal cells have little or no expression of KCNQ1 (14). This is in concert with the observation that treatment of gastric glands and isolated mouse stomach with supermaximal doses (1,000-fold higher than IC₅₀) of a highly specific inhibitor, HMR1556, of KCNQ1 channels still allowed stimulated

Fig. 7. Stimulation-triggered redistribution of KCNJ15 from small vesicles to larger membrane fractions. Rabbit gastric glands were treated with either cimetidine or histamine/IBMX to achieve 2 different physiological states: resting and stimulated. Active acid secretion of the stimulated glands was monitored by [¹⁴C]amino pyrine (AP)-accumulation assay (A). AP ratios for resting and stimulated glands were plotted with error bars representing the standard errors of the means. The ascertained resting and stimulated gastric glands were then homogenized and subjected to differential centrifugation according to the scheme illustrated (B). The same proportion of each fraction was loaded for Western blot analysis with antibodies against H-K-ATPase β subunit, β-actin, and KCNJ15.
acid secretion (15). Kaufhold et al. (15) also reported that most of the KCNQ1 in parietal cells did not translocate to the apical membrane upon stimulation. Therefore, the observation that KCNQ1-knockout mice exhibited complete inhibition of stimulated acid secretion suggested that KCNQ1 might play some additional role in parietal cell biology and development besides apical K+ recycling.

Seidler’s group (15) observed colocalization of Kir4.1 (KCNJ10) and H-K-ATPase on tubulovesicles and the translocation of this channel to apical membrane upon stimulation. However, Fujita et al. (12) reported that Kir4.1 is always localized to the apical membrane. The latter observation is in concert with the fact that K+ channel activity is absent from the tubulovesicles by electrophysiological measurements (18, 28). A possible role for Kir4.1 is also supported by its resistance to low pH (12).

Different from the observation of Fujita et al. (12) with rat, Cuppoletti, Malinowski, and colleagues (5, 19) have reported abundant Kir2.1 (KCNJ2) mRNA and protein in rabbit gastric parietal cells. They also found that Kir2.1 channel activity (open time) can be stimulated by low pH and cAMP, strongly suggesting that Kir2.1 could be involved in stimulated acid secretion.

More recently, the KCl cotransporter KCC4 in parietal cells was found to be associated with a large size membrane fraction called stimulation-associated membranes, but not with a microsomal membrane fraction referred to as tubulovesicles (11). Moreover, both H+ uptake and 36Cl− uptake by the stimulation-associated membrane vesicles were severely inhibited by the KCC inhibitor DIOA, whereas DIOA had virtually no effect on uptake in tubulovesicles. These authors suggested that KCC4 was a resident apical KCl pathway that was supplemented in vivo by additional K+ and Cl− channels recruited to the greatly expanded apical surface after stimulation.

Several groups have hypothesized that multiple K+ channels may cooperate for the task of apical K+ recycling (11, 14, 15). Our observation that both KCNJ15 and KCNQ1 mRNA are highly expressed in the stomach is consistent with this hypothesis. To explain the observation that both KCNQ1 and Kir4.1 (KCNJ10) exist at the same cellular location, Seidler and colleagues (15) suggested that KCNQ1 is important when the parietal cell is depolarized, whereas KCNJ10 is more active when the cell is hyperpolarized, but it is really the channel activation or recruitment that is responsible for the polarization state of the cell. Another explanation for multiple K+ pathways in apical K+ recycling is that a cotransporter such as KCC4 might be needed for basal acid secretion whereas conductive K+ channels might be responsible (recruited) for stimulated acid secretion (11). In fact, it may be naive to expect that a single K+ channel could account for all the requisite properties to accomplish HCl secretion. The channel has to operate at enormous variation of pH and membrane voltage; it has to have characteristics of conductance activation and/or voltage dependence; it is likely to be a subject of membrane recruitment; and there must be flexibility of activation to account for variations in secretory output. There are wide differences among species for observed relative rates of basal vs. stimulated acid secretion (6), and it would be of interest to correlate variations in the apparent levels of different K+ transporters. The discovery of Kir2.1 and KCNJ15 at the apical membrane does not conflict with these possibilities. Yet the reason for so many K+ channels directed toward one general function could be more complicated. For example, considering the inconsistent expression levels of KCNQ1 and KCNJ15 in parietal cells, and the developmentally regulated expression of KCNJ15 in various epithelia (24), it could be hypothesized that the expression levels of certain K+ channels are associated with the development of parietal cells both in early ontogeny and with continued development and differentiation of adult gastric glands.

The data presented here support the participation of another K+ transport candidate, KCNJ15, in membrane recruitment associated with enhanced apical K+ recycling and HCl secretion. At the present time it is not possible to establish which, if not several, of the various candidate transporters participate in the apical K+ conductance that is necessary for K+ recycling and resultant HCl secretion.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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